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# BERYLLIUM "RICKETS"

*By*

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TO PRODUCE experimental rickets in rats it has been found necessary, by almost every worker hitherto, to feed diets having two definite faults, first a gross mineral imbalance between calcium and phosphorus, and second a complete or very nearly complete absence of vitamin D. Human rickets, on the other hand, occurs almost always on diets which are adequate and fairly well balanced as far as their *mineral* salts are concerned, but which are lacking in vitamin D. Since both rat rickets and human rickets are curable by the addition of vitamin D in small quantities to the otherwise rachitogenic diet, they are, very definitely, deficiency diseases.

In beryllium "rickets" however, as will be shown in this paper, we are concerned with an experimental condition which may be developed in animals fed a *normal* diet, containing normal amounts of calcium and phosphorus and adequate vitamin D but to which has been added a relatively small quantity of (basic) beryllium carbonate, a condition which shows many of the characteristics, both histological and chemical, of orthodox experimental rat rickets[1] and, if the quantity of beryllium in the diet is sufficient, shows them in a grossly exaggerated form. Though we have not explored all the possibilities in this direction it appears that the severity of the rickets can be graduated with considerable nicety by adding more or less of the beryllium salt to the normal diet.

In investigating the effect of certain activators and inhibitors on mammalian phosphatase one of us found, some two years ago, that $Be^{++}$ like $Ca^{++}$ was occasionally strongly activating to the enzyme. As is indicated

[1] By "experimental rat rickets" we mean the type of bone lesion produced by feeding rats on high Ca–low P diets, such as those of McCollum (3143), Steenbock (2965) or Osborne and Mendel, and not the type of change, usually considered to be rather osteoporotic than rachitic, produced by a high P–low Ca diet.

by the position of both elements in Group 2 of the periodic table, beryllium has many points of chemical resemblance to calcium; for present purposes it is only necessary to mention that, in addition to an insoluble hydroxide and carbonate, it forms phosphates which, like the corresponding phosphates of Ca, are very insoluble at neutrality and on the alkaline side of it, but which, unlike the phosphates of calcium, are also relatively insoluble even in fairly acid media. In view of this last property, it occurred to us that interesting results might follow the introduction of beryllium salts into the diet of experimental animals, particularly since a preliminary trial showed that, when mixed with either a normal or a rachitogenic diet, beryllium carbonate was well tolerated by rats.

We first found (1) that a very severe type of rickets followed when the McCollum or the Steenbock rachitogenic diet, modified by replacing the calcium carbonate by its equivalent of beryllium carbonate, was given to growing rats (21 to 24 days old when placed on the diet). More recently we have added the beryllium salt in varying amount to the normal stock diet (Bills') and have found that when quantities of the order of 0.25 per cent or more are added, the diet produces severe rickets in growing rats. With quantities of beryllium carbonate even as low as 0.125 per cent of the diet, definite rickets ensues in about three weeks time.

## Experimental

(a) *Gross changes produced by beryllium carbonate.*—The rate of increase of growth of the rats was, in all the beryllium carbonate-containing diets, greatly diminished, the effect being as marked when the salt was added to the normal as to the modified Steenbock or McCollum diets. Animals soon showed much less activity than normal controls; after about a fortnight, with the larger quantities of beryllium, and somewhat longer with the lesser quantities, the gait became waddling and the back arched. After 24–28 days on the diet, the animals which were now in every case much under the normal weight for their age, were killed, and the bones and other tissues subjected to macroscopic, x-ray, histological, and chemical examination. The thorax showed typical rachitic deformities, with very marked swelling of the costochondral junctions. All the bones were very thin and fragile. The organs appeared to be normal; there was a characteristic color of most of the soft tissues in the animals which had received the greater percentages of beryllium, probably due to the anemia which was found to be present.

(b) *X-ray Examination.*—The x-ray photographs, particularly of those

animals which had had 1 per cent or more of the beryllium salt added to their (otherwise normal) diet, showed bones of a density considerably below that of the controls, with almost complete lack of calcification in the epiphyseal cartilage. Since the atomic weight of beryllium is so low (9.1) it is of course possible that even if Be salts had been deposited in the bones they would not have given an x-ray shadow, but as will be mentioned later, chemical examination of the bone ash failed to reveal any detectable quantity of Be. With the lower quantities of the salt (0.5 per cent) added to the normal diet, the appearance of the upper end of the tibiae was somewhat similar to that of rats with ordinary severe experimental (low P) rickets, but the heads of the femora were almost completely uncalcified in the former case. With still smaller quantities of beryllium carbonate (0.25 and 0.125 per cent) the "rickets," though quite definite, was rather less severe than that usually met with after feeding the Steenbock or McCollum rachitogenic diet for the same period.

(c) *Histological findings.*—For study it was decided to section the lower end of the femur and the adjoining portion of the tibia. The use of both bones had the advantage that owing to the slight difference in the time of appearance of the centres of ossification of the two bones, a comparison was possible of the effect of the nutritional disturbances on bones in slightly different stages of development.

The quantity of beryllium carbonate given varied in amounts from 3 per cent to 0.12 per cent of the diet. X-ray photographs indicated the most marked alteration from the normal in the deposit of bone salts in the epiphyseal region of the bones of rats which received the maximum dosage.

Sections were cut in the sagittal plane. Rats under fifty days old which received 0.12 per cent (Plate 1, Fig. 2) and 0.25 per cent (Plate 1, Fig. 3) of the beryllium salt, and rats seventy-six days old which received 2 per cent of the salt, showed a band of calcification extending wholly or partially across the bone in the provisional zone and also small deposits of bone salts dotted here and there in the osteoid tissue.

In many cases the trabeculae of the diaphysis, and also the cortex, indicated mineral deposition much below normal.

In all cases there was an increase of intercellular matrix of the epiphysis, especially in the proliferation zone. In a few instances there was an invagination of the cartilage (including the proliferative zone) into the secondary centre of ossification. There was also a marked increase marginally in the amount of cartilage of the head, often extending shaftwards over the margins of the columnar epiphyseal cartilage.

The epiphysis was greatly deepened, yet the columnar arrangement was usually well maintained. The proliferative zone of fusiform and flattened cells so well described by Dodds (2) was hardly altered except for the increase of intercolumnar matrix. This zone was from 20–25 cells deep both in normal and in beryllium-fed rats. The extraordinary variation observed was the presence of long strings of globular cartilage cells (Plate 1, Fig. 9B). Whereas these columns normally contain five or six, in beryllium "rickets" they extended to fifty, sixty or even eighty cells in length. With the exception of the distal fifteen or twenty the columnar arrangement was well maintained. Many cells in the deeper portion of the metaphysis became flattened and fusiform in shape. The cytoplasm of some of these stained deeply with hematoxylin and exhibited vacuolation, while the nucleus retained its affinity for eosin. On the other hand, at the junction of the shaft and the metaphysis these cells lost this affinity for hematoxylin, the cytoplasm being pink and the nucleus outlined in red with a granular content. Mitotic figures were numerous among these latter cells and frequently as many as six daughter cells could be seen still held within the parent capsule. In the tibia in animals showing the gravest disturbance of bone development, cartilage elements involved the whole of the central zone of the metaphysis and extended to the metaphyseal margins, both above and below. Marginally the advent of capillaries was associated with the conversion of the cartilage into osteoid tissue. This tissue was very irregular in outline with vascularity much more in evidence along its cartilaginous margin. Occasionally small groups of globular cartilage cells were incorporated within the osteoid tissue, bone salts being rarely present in this tissue.

The trabeculae of the diaphysis were usually narrow and less in evidence toward the centre, while at the margin they were numerous and regular.

Centrally the bone marrow extended in most cases almost to the level which had been occupied by the provisional zone of calcification at the time when beryllium was added to the diet. Usually small and occasionally large vacuoles were observed in this marrow. This vacuolation was well marked in rats which had received the Steenbock diet without added $CaCO_3$.

In Plate 1 will be found photographs of bone sections, illustrating certain of the points just mentioned, made from the tibiae and femora of rats with beryllium "rickets" of varying severity.

(d) *Chemical examination.*—The blood and samples of the other tissues, taken from all of the animals in a group (usually six), were pooled for

analyses. Precautions were taken to avoid, as far as possible, errors which might arise from rapid postmortem changes in the tissues which would affect, for example, the inorganic phosphate content of the blood, or the phosphoric ester content of the liver.

Typical chemical findings are summarized in Tables I and II.

TABLE I
CHEMICAL FINDINGS IN BERYLLIUM "RICKETS"

| Expt. | Diet | Bone ash per cent[1] | Inorg. P in plasma mg. | Phosphatase in | | | Remarks |
|---|---|---|---|---|---|---|---|
| | | | | plasma units | bone units | kidney units | |
| 916 | Normal | 56 | 6.9 | 0.65 | 31 | — | X-ray normal |
| | Steenbock 2965 without $CaCO_3$ | 48 | 5.5 | 0.86 | 37 | — | X-ray no rickets density diminished |
| | Steenbock 2965 no $CaCO_3$ but 2% $BeCO_3$ | 43 | 0.65 | 0.84 | 33 | — | Rickets |
| | Ditto 2 drops viosterol daily | 43 | 0.84 | 0.48 | 21 | — | Rickets |
| | Ditto ultraviolet light daily | 41 | 0.67 | 0.64 | 34 | — | Rickets |
| | Ditto 2 drops C.l.o. daily | 42 | 0.65 | 0.64 | 24 | — | Rickets |
| 926 | Normal diet | 59 | 8.6 | — | 23 | 49 | X-ray normal Serum Ca. 9.85 |
| | Steenbock 2965 no $CaCO_3$ but 1% $BeCO_3$ | 42 | 1.3 | — | 24 | 21 | Rickets Serum Ca. 10.0 |
| | Steenbock 2965 no $CaCO_3$ but 1% $BeCO_3$ | 44 | 1.2 | — | 31 | 12 | Rickets, not very typical |

[1] Percentage of dry, fat-free wt.

## DISCUSSION

One of the most striking features of experimental rickets and of the majority of cases of human rickets, is that the inorganic phosphorus content of the blood or plasma is low. In experimental rickets in rats, if the diet be properly planned, in spite of the low quantity of phosphorus ingested, and although there is defective deposition of both Ca and P in the growing bones, the growth of the soft tissues and the increase in total weight of the animal occurs at only a little below the normal rate. This points to fairly

TABLE II

CHEMICAL FINDINGS IN BERYLLIUM "RICKETS"

| Expt. | Diet | Bone ash percent | Inorg. P. in plasma mg. | Phosphatase in | | | Liver per 100 gms. | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | plasma units | bone units | kidney units | inorg. P mg. | acid- sol. P mg. | ester P mg. | |
| 17 | Normal | — | 5.1 | 0.66 | 28 | 46 | 42 | 126 | 84 | X-ray normal |
| | Normal+0.5% $BeCO_3$ | — | 2.7 | 0.56 | 26 | 33 | 43 | 90 | 47 | Rickets |
| | Normal+1.0% $BeCO_3$ | — | 2.2 | 0.47 | 34 | 23 | 44 | 106 | 62 | Rickets |
| | Normal+2.0% $BeCO_3$ | — | 1.5 | 0.54 | 29 | 13 | 45 | 96 | 51 | Rickets |
| 154 | Normal | 57 | 7.9 | — | — | 36 | 49 | 134 | 83 | Normal |
| | Normal+0.5% $BeCO_3$[1] | 35 | 0.78 | — | — | 20 | 52 | 99 | 47 | X-ray rickets like Steenbock |
| | Normal+1.% $BeCO_3$ | 29 | 0.47 | — | — | 17 | 51 | 107 | 56 | Severe rickets |
| | Normal+2.% $BeCO_3$ | 31 | 0.31 | — | — | 11 | 52 | 89 | 37 | Severe rickets |
| | Normal+2.% $BeCO_3$+cod liver oil | 32 | 0.27 | — | — | 12 | 51 | 91 | 40 | Severe rickets |
| | Normal+2.% $BeCO_3$+viosterol | 28 | 0.25 | — | — | 12 | 50 | 94 | 44 | Severe rickets |
| 166 | Normal | 56 | 7.0 | 0.50 | — | 36 | 42 | 132 | 90 | Normal |
| | Normal+0.125% $BeCO_3$ | 41 | 3.0 | 0.42 | — | 37 | 43 | 100 | 57 | Rickets |
| | Normal+0.25% $BeCO_3$ | 38 | 2.9 | 0.41 | — | 35 | 43 | 105 | 62 | Rickets |
| | Steenbock 2965 | 31 | 2.5 | 0.67 | — | — | 47 | 88 | 41 | Severe rickets |
| | Steenbock 2965+cod liver oil | 42 | 2.7 | 0.79 | — | — | 51 | 99 | 48 | X-ray normal |

[1] The serum calcium determined in two groups only, appeared to be normal.

efficient uptake from the intestine of the ingested phosphate, of which a considerable quantity is required for the growth of the soft tissues, particularly muscle and liver. It is to be remarked that in case of phosphate shortage in the diet, these tissues, in absence of vitamin D, appear to have first claim on the available phosphate supply, the demands of the bones remaining unsatisfied.

From Table II it will be observed that in many cases of beryllium rickets the inorganic phosphate content of the plasma is reduced to extremely low values, which are, as far as we can discover, the lowest plasma phosphate figures ever recorded in the literature. In some of the beryllium groups inorganic phosphate almost disappears from the plasma, and can only be estimated by the device of adding a small quantity of phosphate to the protein-free filtrate before estimation and subtracting the added quantity afterward. There is evidently a very acute shortage of this radical in the blood, which it seems legitimate to correlate on the one hand with the very poor growth and complete lack of calcification, and on the other with the extreme insolubility of beryllium phosphate at intestinal pH, or even at the pH of the gastric contents. Any phosphate going into solution in the fluids of the gut, or liberated by enzymic hydrolysis of phosphoric esters, will be immediately precipitated by the beryllium ions resulting from the solution of the basic carbonate in the gastric juice, and will thus be rendered unavailable for absorption through the gut wall. This extreme insolubility of beryllium phosphate even in acid range of pH is indicated by the following experiment. To a number of tubes containing 5 cc. phthalate buffers at pH's ranging from 2.2 to 5.4 was added 1 cc. of M/10 $BeCl_2$ solution, adjusted to $pH_4$. To a similar series of tubes was added the same quantity of M/10 $CaCl_2$ also adjusted to $pH_4$. Then to all the tubes was added 0.5 cc. of approximately M/10 potassium phosphate adjusted with dilute NaOH to the same pH as the buffers already in the tubes.

In the calcium tubes, a slight precipitate appeared at pH 5.4 but none at any lower pH. In the beryllium tubes, there was a large precipitate in the pH 5.4 tubes, and a precipitate in slightly diminishing quantities down to pH 2.6. The tube at pH 2.2 remained, however, quite clear. On filtering and carrying out phosphorus determinations on the filtrate, the following quantities of inorganic P were found

| pH of tube: | below 1 | 2.2 | 2.6 | 3.0 | 3.4 | 3.8 | 4.2 | 4.6 | 5.0 | 5.4 |
|---|---|---|---|---|---|---|---|---|---|---|
| mg. P per cc. of filtrate | 1.64 | 1.64 | 1.36 | 1.03 | 0.86 | 0.51 | 0.38 | 0.26 | 0.20 | 0.14 |

Thus while under the above conditions there is no precipitate whatever of calcium phosphate at a pH of 5.0 or less, at this pH, and at pH's considerably more acid down to below 4, by far the greater part of the inorganic phosphate is precipitated by beryllium. Had the beryllium been present in excess, even larger portions of the phosphate would have been taken out of solution.

It has been found that at neutrality the precipitation of inorganic phosphate by slight excess of beryllium chloride is quantitative.

The amount of basic beryllium carbonate required to be added to a normal diet to bring about this acute bone dystrophy is only 0.5 per cent. Less than this amount (0.25 per cent and even 0.125 per cent) will produce definite rachitic changes (see Plate 1, Figs. 2 and 3). The normal stock diet we have used contains Ca 0.40 per cent and P 0.42 per cent, varying a little with the quality of the casein used. The basic carbonate employed by us contains 68 per cent $BeCO_3$ and 32 per cent BeO, or about 20 per cent of Be. To combine with the whole of the phosphorus of the diet to form $BeHPO_4$ would therefore require the addition to the diet of some 0.6 per cent, or to form $Be(H_2PO_4)_2$ the addition to the diet of 0.3 per cent, of the basic carbonate. In accordance with this calculation it is found that in the bones of young animals which have been fed diets containing 1.0 per cent or above of the carbonate there is absolutely no evidence of calcification (x-ray) or phosphorification (von Kossa), with 0.5 per cent of the beryllium there is practically no calcification, and only with 0.25 per cent or less is any new bone salt laid down during the period of beryllium feeding. Even with 0.125 per cent of the carbonate added to the normal diet the inorganic phosphate concentration of the plasma is reduced, after three weeks feeding, to 2.4 mgm. P per 100 cc. below the usual rachitic level on the Steenbock or McCollum rachitogenic diet.

This question of the effect on the bones of the addition to a normal diet of very small quantities of beryllium salts is being investigated further, and it is not impossible that it may be due to other properties of beryllium than its power to precipitate phosphates quantitatively from slightly acid solutions.

Three other points of some interest arise out of the data presented in Table II. The first is that despite the astonishingly low plasma inorganic phosphate, the inorganic phosphate of the liver in beryllium rickets remains at its normal level; the second is that the phosphoric ester (acid-soluble organic phosphorus) of the liver is definitely diminished in this experimental condition (this is also the case in simple experimental rickets

in rats)[2]; the third is the progressive diminution in the phosphatase of the kidney tissue with the increasing beryllium carbonate in the diet. This last effect is not a necessary sequel to the rachitic condition, since it has been found (3) that even in severe "Steenbock" or "McCollum" rickets the phosphatase of the kidney is only slightly diminished. How this large effect is produced in beryllium rickets is quite unknown.

Of the influence on nutrition of the metals belonging to Group 2 in the periodic system which are not toxic on ingestion, that of strontium was investigated some years ago by Konig (4), by Stoelzner (5) and by Lehnerdt (6) and the results have been confirmed and extended by Shipley, Park, McCollum, and Kinney (7). All agree that strontium will not replace calcium in the diet, but if $Sr_3(PO_4)_2$ is fed to puppies or young rabbits or rats it produces a type of bone dystrophy characterized by an excessive production of osteoid and by the presence of considerable quantities of Sr in the bones. Shipley and collaborators found also that the administration of cod liver oil in antirachitic doses does not prevent the onset of this type of "rickets" (termed by Lehnerdt "strontium sclerosis").

While these experiments with Sr are not strictly comparable with our own with Be, they have in common the finding that strontium "sclerosis" and beryllium "rickets" (at least when produced by 2 per cent beryllium carbonate in the "normal" diet) are both quite unamenable to treatment with cod liver oil. In addition, beryllium "rickets" is neither prevented nor cured by liberal doses of irradiated ergosterol or direct irradiation of the experimental animals. It was noticed, however, that both the cod liver oil and the irradiated ergosterol markedly increased the food intake of the treated animals. On the whole, in spite of the fact that growth was greatly interfered with, even on a 0.12 per cent beryllium carbonate diet, the food intake was good, and other things being normal, the amount eaten should have given good growth. The rats appeared not to suffer from intestinal disorder, and the feces were usually of normal consistency.

Semi-quantitative tests using (a) a sodium peroxide method of C. L. Parsons, described by Mellor (8); (b) a simple method founded on the relative insolubility of beryllium and the relative solubility of calcium and magnesium phosphates at pH 5.4, indicates that there is not, in any of the specimens of bone ash derived from the rats fed on diets containing beryllium, more than one part of Be to 250 parts of Ca. Actually there is probably considerably less than this. Thus there is no storage of Be in the bones on a Be-rich diet.

[2] To be published.

The results of these experiments appear to us to be consonant with the view that experimental rickets is primarily due to the deficient absorption of phosphate from the intestine (which view has received strong support recently from the work of Heymann (9)), and that the severity of the rickets depends on the extent of this deficiency.

Further work on the nutritional effects of beryllium salts is in progress.

## Summary

By replacing the calcium carbonate in Steenbock's rachitogenic diet 2965 with an equivalent amount of beryllium carbonate, or by adding small quantities of this substance to the *normal stock diet* (Bills'), bone lesions which have certain similarities to rickets may readily be produced in young rats, the severity of the lesions bearing an approximate relationship to the amount of the beryllium salt in the diet. The percentage of ash in the bones is much diminished. Both x-ray photographs and histological sections reveal almost complete failure of mineral deposition in the metaphysis, even immediately proximal to the epiphyseal disc, and reduced amounts of mineral salts in the trabeculae and cortex of the tibia. Other long bones are similarly affected. The depth of the metaphysis is comparable to that exhibited in severe Steenbock rickets. The maintenance of the columnar arrangement of the cartilage cells in the metaphysis is distinctive of beryllium "rickets" in the rat.

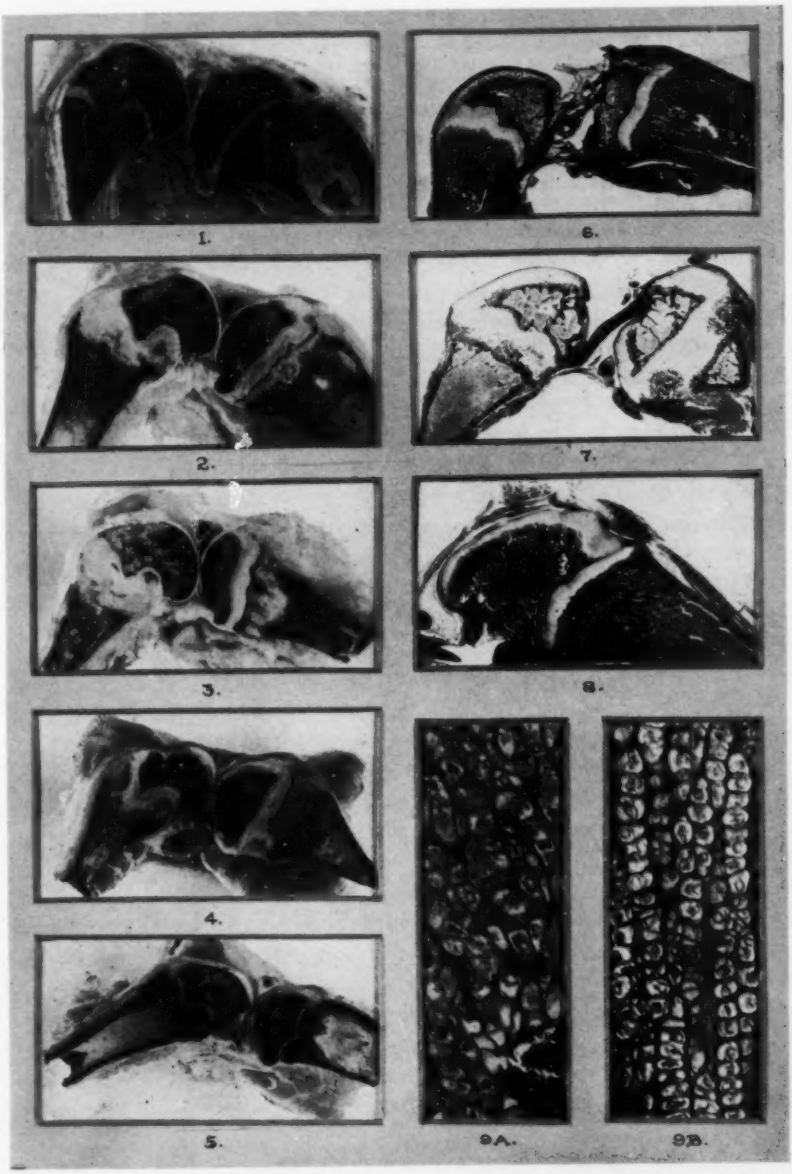
The inorganic phosphorus content of the blood plasma is very much reduced, and the acid-soluble phosphoric esters of the liver are diminished in quantity. The phosphatase content of the kidney is markedly lowered. Beryllium does not appear to be deposited in appreciable quantities in the bones. This type of bone lesion is not preventable by cod liver oil nor by irradiated ergosterol administration, nor is it amenable to the anti-rachitic influence of ultraviolet light.

Plate 1

1. Von Kossa, Normal.
2. Von Kossa, Beryllium "rickets" 0.12 per cent beryllium in normal diet.
3. Von Kossa, Beryllium "rickets" 0.25 per cent beryllium in normal diet.
4. Von Kossa, Steenbock rickets (2965).
5. Von Kossa, High P "rickets."
6. Mallory, Normal.
7. Mallory, Beryllium "rickets" (3 per cent Be to Steenbock diet).
8. Mallory, Beryllium "rickets" (0.12 per cent Be to normal diet).

9A. Mallory, Rickets on Steenbock diet (2965).

9B. Rickets on Steenbock diet (2965) but having 2 per cent Beryllium carbonate in place of 3 per cent Calcium carbonate (showing relative regularity of cartilage proliferation in latter type of rickets).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# STUDIES ON THE RELATION OF DIET TO GOITER*

## I. A DIETARY TECHNIC FOR THE STUDY OF GOITER IN THE RAT†

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THE work of Remington and associates (1, 2) on iodine in human environment in South Carolina, as compared with that of McClendon (3) for states of the Northwest, coupled with the data collected by Olesen (4) as to incidence of thyroid enlargement in various parts of the United States, has aided in substantiating the correlation between an adequate iodine supply and the normal development of the thyroid gland, previously indicated by work in Europe and New Zealand. The examination of 17,600 school children of South Carolina, with a goiter incidence of only 3.8 per cent, as reported by Hayne (5) has added further weight to the contention that goiter is rare in this area, and that its scarcity can be correlated with an adequate supply of iodine.

The generally accepted theory that endemic goiter is due to an iodine lack rests on such chemical and clinical observations as the above, rather than on direct experimental evidence, which up to this time has been very meager, and for several reasons. First, methods for the reasonably accurate estimation of the minute amounts of iodine involved have not until recently been perfected, and second, a dependable dietary technic for goiter production in an experimental animal has not been devised. For these reasons our attention was first directed toward the development of a dietary technic which could be depended upon to produce marked thyroid enlargement and other symptoms referable to simple goiter in the rat so that later we might be able to carry out various studies on goiter.

A review of the literature revealed that several investigators (6-14) have carried out goiter studies using the rat as the experimental animal. A study

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of the various diets used by these workers to produce goiter indicated to us that they were not suitable, for several reasons. First of all, in some cases the production of goiter was brought about by means of rations which were deficient in one or more of the recognized dietary essentials other than iodine, while in certain instances the production of goiter was frequently accompanied by various types of infections which often resulted in the death of the experimental animals. Some workers were able to produce thyroid enlargement in some rats but not in others, while other investigators were able to produce only slight glandular enlargement, due presumbaly to the fact that the diet was only slightly deficient in iodine. With a few exceptions, iodine estimations on the diets were not carried out, thereby making correlations between the iodine intake, the iodine concentration in the thyroid, and the goitrogenic activity of the diet impossible. In the few instances where estimations of the iodine content of the diets were made, the values were obtained by analytical methods which are open to criticism. Some workers have concluded from their experiments that a lack or deficiency of iodine is not the cause of goiter, but such a contention has not been supported by a determination of the iodine content of the diets employed. A further study of the literature revealed the important fact that no investigator has apparently been able to bring about consistently a marked thyroid enlargement in the rat.

Since in our contemplated goiter studies, we were anxious to employ a goitrogenic diet of known iodine content which could be relied upon to produce consistently a marked thyroid enlargement in a short period of time, it was evident from the above critical study of the literature that we would be forced to devise such a suitable dietary technic ourselves.

In the adoption of a diet low in iodine for the study of goiter, we were guided principally by the findings of Krauss and Monroe (11) who discovered that the well-known rickets-producing ration of Steenbock and Black (15) produced, simultaneously with the rickets, enlarged thyroid glands in the rat. This diet has the following composition: yellow corn, 76 parts; wheat gluten, 20 parts; calcium carbonate, 3.0 parts; and sodium chloride, 1.0 part. These investigators, however, did not make a detailed study of the influence of this ration on the thyroid gland. Histological studies showing the effect of the goitrogenic ration on the structure of the thyroid gland were not made. Since the iodine content of the goitrogenic ration was not determined, Krauss and Monroe were unable to correlate the iodine content of this diet with the iodine content of the thyroid and in turn with the extent of thyroid enlargement. This information was

particularly desirable for our later studies on the determination of the minimum iodine requirement of the rat. These investigators also failed to ascertain conclusively whether the presence or absence of rickets and hence the presence or absence of the antirachitic vitamin D influenced the goitrogenic capacity of the diet. Both Mellanby (16) and Murray (17) have reported the occurrence of goiter along with rickets in the dog. Further, Krauss and Monroe report the average thyroid weight of only four normal stock rats. Accordingly, sufficient data to establish the normal thyroid weight of the rat were not available for comparison in calculating the extent of thyroid enlargement in the goitrous animals. With the above considerations in mind, we have made an intensive study of the influence of the Steenbock diet on the thyroid gland of the rat, and, as a result, have developed a dietary technic suitable for the study of goiter.

## Experimental

The rats used in these studies were bred in our laboratory on an adequate stock ration which has been used in the production of many healthy generations of young for this and other experimental work. The composition of this stock diet is as follows: whole wheat (ground), 59.8 parts; whole milk powder,[1] 29.9 parts; Swift's meat scrap (55 per cent protein), 9.1 parts, and sodium chloride, 1.2 parts. In most of the studies, ten animals were placed on each diet so as better to control the factor of biological variation as well as to yield sufficient thyroid material to facilitate an accurate determination of the average iodine content of the glands in each group. The iodine content of all diets and food materials was determined by the methods employed in this laboratory (18).

At the end of the experimental feeding period, the rats were killed by means of chloroform. The thyroids were then removed and weighed immediately. In order to make fair comparisons of the effects of different diets on the weight of the thyroid, calculation of the fresh weight of the gland in mg. per 100 grams body weight was made. Next, the glands of all rats on the same diet were pooled and dried to constant weight at 80°. The iodine content of the dried composited glands was then determined by a modification of the micro-McClendon method (19). In some experiments, representative glands were saved for histological study.

In preliminary experiments, using 82 rats, we were able to confirm the findings of Krauss and Monroe (11) and of Clausen (20) relative to the marked thyroid enlargement produced by the Steenbock ration. The

[1] Klim.

following additional facts were also ascertained in these preliminary experiments:

(a) Iodine (4 to 5γ daily[2] as KI) reduces thyroid enlargement produced by the Steenbock diet, increasing simultaneously the iodine content of the gland.

(b) Goitrous glands were found to have a dark red color while normal glands were pale pink.

(c) Goitrous glands were low in iodine and in dry matter content.

(d) Rats bred on a high iodine stock ration were able to store iodine which subsequently retarded marked glandular enlargement when the animals were placed on the goitrogenic ration.

(e) Goiter develops in the presence or in the absence of rickets. A similar finding has been reported recently by Thompson (21).

(f) Although it was assumed (22–24) that the yellow corn of the ration furnished an adequate supply of vitamin A, the further addition of carotene to the diet did not influence glandular enlargement. McCarrison (10, 25) and Drennan, Malcolm and Cox (13) have made studies on avitaminosis A as a contributary cause of goiter.

(g) Anemia is not a concurrent manifestation of goiter. Anemia sometimes accompanies goiter in the human (26,27). However, ten goitrous rats showed normal hemoglobin values ranging from 14.5–17.1 gm. per 100 cc. of blood. The iron and copper content of the diet was shown by analysis to be adequate (50).

Based on our findings in the preliminary experiments, we adopted a modification of the Steenbock rachitic diet to be used as a standard goitrogenic diet in all subsequent experiments. To each 100 gm. of the Steenbock ration, we added 0.2 gm. of irradiated yeast[3] having a vitamin D potency fifteen times that of standard cod liver oil. We shall refer to this modified goiter-producing diet as Diet GP. Since we had found that a marked thyroid enlargement took place on Diet GP in the short period of 35 days, we decided to use this time interval as the length of the experimental period in subsequent experiments all of which were of the preventive type. Various batches of Diet GP were found to be low in iodine and to have an average iodine content of 15 γ per kilo.

With the aid of the goitrogenic Diet GP, we have begun the study of

[2] The Greek letter *gamma* is used for 0.001 milligram.

[3] Cod liver oil could not be used as a source of vitamin D because of its high iodine content. The irradiated yeast was furnished us through the courtesy of the Fleischmann Laboratories, New York City.

many problems involving goiter. In the course of these experiments, it was deemed necessary to compare the gross and microscopic appearance, weight, iodine content, dry matter content, etc., of the thyroids of rats fed Diet GP with similar data obtained for animals fed either the stock ration or the goitrogenic diet to which iodine was added (Diet GPI[4]). With this information, we have been able to establish data for the *normal* thyroid from which the extent of glandular enlargement, loss in iodine content, change in the color, etc. resulting from the goiter-producing Diet GP were ascertained. Data on the three above mentioned diets have been collected from five series of experiments and are shown in Table I. Table II gives the average values for each diet shown in Table I and contains in addition data for the thyroids of stock rats at the start of the experimental period. The values given in Table II represent the averages of 1.—20 stock rats at the start of the experimental period, 2.—39 rats on the stock diet, 3.—193 rats on Diet GP and 4.—24 rats on Diet GPI.

A study of Table II reveals many striking facts which can best be brought out when discussed under various headings.

(a) *Appearance of the thyroid gland.*—Rats receiving Diet GP yielded enlarged thyroids, dark red in color, with considerable engorgement and distension of the blood vessels leading to the gland. In contrast to this finding, glands of rats on Diet GPI were pale pink in color with no engorgement of the blood vessels leading to the gland, while rats fed the stock ration yielded similar results except that the gland tended to be red rather than pink in color, indicating possibly that the stock ration contains an amount of iodine slightly below the requirement of the rat.

(b) *Histological findings.*—Marked hyperplasia together with little or no iodine-containing colloid was obtained on Diet GP. Thompson (21) also reports a similar finding using the same ration. On the other hand, Diet GPI yielded glands with a normal histological appearance containing considerable colloid, while the stock ration yielded glands that were nearly normal and contained less colloid than the glands of rats fed Diet GPI. Figure I shows the histological appearance of three representative rats (litter mates) fed the three rations. A report[5] on the histological findings in the thyroids of these rats is given below:

[4] The iodine was carefully incorporated in the ration by evaporating a solution of KI on a small portion of the ration. This material was then thoroughly mixed with the remainder of the diet.

[5] We are indebted to Dr. Kenneth M. Lynch, head of the Department of Pathology, Medical College of the State of South Carolina, for the preparation and interpretation of the histological sections of the rat thyroids.

TABLE I

SHOWING THE PRODUCTION OF GOITER IN THE RAT AND ITS PREVENTION BY THE ADMINISTRATION OF IODINE

(All rats started at approximately 60 gm. body weight* and fed experimental diets for 35 days)

| Series No. | Iodine content of diet | Iodine intake | | No. of rats | Initial and final body wts. | Dry matter in thyroid | Average thyroid weight | | | | Iodine conc. in gland | | Total iodine in each gland | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Fresh | | Dry | | | | | |
| | | Av. daily | Av. total | | | | Actual | per 100 gm. body wt. | Actual | per 100 gm. body wt. | Dry basis | Fresh basis | Actual | per 100 gm. body wt. |
| | γ/kilo | γ | γ | | gm. | % | mg. | mg. | mg. | mg. | % | % | γ | γ |
| *Goitrogenic Diet—GP* | | | | | | | | | | | | | | |
| I | 15 | .13 | 4.6 | 16 | 60–90 | 19.5 | 69.1 (30.3–109.0) | 78.1±2.9 (34.1–126.0) | 13.5 | 15.2 | .0064 | .0013 | 0.86 | 0.97 |
| II | 17 | .14 | 4.9 | 45 | 62–109 | 18.5 | 50.1 (25.8–88.6) | 46.1±1.2 (24.1–72.7) | 9.3 | 8.5 | .0110 | .0020 | 1.02 | 0.94 |
| III | 14 | .13 | 4.6 | 29 | 60–102 | 19.7 | 42.6 (24.4–78.7) | 41.8±1.9 (25.0–82.0) | 8.4 | 8.2 | .0102 | .0020 | 0.86 | 0.84 |
| IV | 14 | .14 | 4.9 | 53 | 63–112 | 18.6 | 69.3 (35.5–117.1) | 62.1±3.2 (34.7–102.8) | 12.9 | 11.6 | .0060 | .0011 | 0.77 | 0.70 |
| V | 15 | .14 | 4.9 | 50 | 60–123 | 19.4 | 53.6 (32.6–93.6) | 43.5±2.3 (26.1–63.5) | 10.4 | 8.4 | .0076 | .0015 | 0.79 | 0.64 |

*Goitrogenic Diet Containing Added Iodine†—GPI*

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| II | Iodized $H_2O$ | ‡ | — | 4 | 61–112 | 26.5 | 13.1 (11.3–15.8) | 11.8± .16 (11.2–12.3) | 3.5 | 3.1 | .2761 | .0732 | 9.66 | 8.53 |
| III | 400 | 3.72 | 130.2 | 10 | 62–106 | 28.0 | 13.1 (10.2–17.4) | 12.4± .25 (10.5–13.5) | 3.7 | 3.5 | .3038 | .0851 | 11.16 | 10.40 |
| IV | 400 | 3.72 | 130.2 | 10 | 64–115 | 28.3 | 14.9 (11.4–17.7) | 13.1± .36 (10.5–16.0) | 4.2 | 3.7 | .2275 | .0644 | 9.60 | 8.42 |
| | | | | | | | *Stock Diet* | | | | | | | |
| II | 60 | 0.81 | 28.4 | 19 | 61–193 | 24.0 | 22.9 (17.4–33.2) | 11.9± .28 (9.0–15.4) | 5.5 | 2.9 | .0666 | .0160 | 3.66 | 1.93 |
| III | 47 | 0.51 | 17.9 | 10 | 60–160 | 21.2 | 23.4 (15.9–36.0) | 14.5± .53 (10.9–18.6) | 5.0 | 3.2 | .0518 | .0110 | 2.59 | 1.63 |
| IV | 72 | 0.82 | 28.7 | 10 | 58–169 | 24.0 | 22.3 (17.4–35.4) | 13.3± .44 (9.8–16.3) | 5.4 | 3.2 | .0691 | .0166 | 3.50 | 2.21 |

* Average age = 27 days.

† As KI.

‡ Approximately 4 $\gamma$ daily intake of iodine per rat in the form of iodized $H_2O$ containing KI.

TABLE II

A SUMMARY OF DATA SHOWING THE PRODUCTION OF GOITER IN THE RAT AND ITS PREVENTION BY THE ADMINISTRATION OF IODINE
(Rats started at approximately 60 gm. body weight* and fed experimental diets for 35 days)

| Diet | Iodine content of diet | Iodine Intake | | No. of rats | Initial and final body wts. | Dry matter in thyroid | Average thyroid weight | | | | Iodine conc. in gland | | Total iodine in each gland | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Fresh | | Dry | | | | | |
| | | Av. daily | Av. total | | | | Actual | per 100 gm. body wt. | Actual | per 100 gm. body wt. | Dry basis | Fresh basis | Actual | per 100 gm. body wt. |
| | γ/kilo. | γ† | γ | | gm. | % | mg. | mg. | mg. | mg. | % | % | γ | γ |
| Stock | 47–72 | — | — | 20 | 62 | 25.6 | 10.2 (8.9–13.8) | 16.4± .32 (14.1–21.2) | 2.6 | 4.2 | .0428 | .0110 | 1.11 | 1.80 |
| Stock | 47–72 | 0.74 | 25.9 | 39 | 60–178 | 23.3 | 22.9 (15.9–36.0) | 12.9± .25 (9.0–18.6) | 5.3 | 3.0 | .0634 | .0147 | 3.36 | 1.90 |
| GP | 15 | 0.14 | 4.9 | 193 | 62–109 | 19.0 | 58.0 (24.4–117.1) | 53.2± .92 (24.1–126.0) | 10.8 | 9.9 | .0083 | .0016 | 0.88 | 0.82 |
| GPI‡ | 400 | 3.72 | 130.2 | 24 | 63–111 | 28.0 | 13.8 (10.2–17.7) | 12.6± .19 (10.5–16.0) | 3.9 | 3.5 | .2671 | .0745 | 10.28 | 9.26 |

* Average age = 27 days.
† 1 mg. = 1000 γ.
‡ Containing added iodine as KI.

*Rat 551. Diet GP.*—Blood vessels engorged. No smooth colloid. Lumen of acini small. Content of acini stringy and granular. Lining cells large, with granular reticulated cytoplasm. Nuclei large and vesicular. Some intra-acinar papillary growth. Apparently a very active gland, with little colloid accumulation.

*Rat 553. Diet GPI.*—Gland normal. Acini filled with colloid, quite vacuolated, lining cells flattened cuboidal.

*Rat 554. Stock Diet.*—Gland near normal. Most acini contain smooth colloid and are lined by low or flattened cells. Some acini contain a granular material and the lining cells are large, cytoplasm granular, nucleus large and vesicular, cell outlines indistinct.

Marine (28) has described in detail the steps in the process of hyperplasia in the human thyroid. The histological findings in the goitrous glands of the rat are very similar to those reported by Marine for the human.

(c) *Comparison of fresh thyroid weights.*—The thyroid weights for the 193 rats receiving Diet GP yielded an average value of 53.2 ± .92 mg. per 100 gm. body weight, with 24.1–126.0 mg. as the extremes of enlargement. Diet GPI yielded an average thyroid weight of 12.6 ± .19 mg. per 100 gm. body weight with a range of 10.5–16.0 mg., while the stock ration[6] gave an average gland weight of 12.9 ± .25 mg. per 100 gm. body weight with 9.0–18.6 mg. as the extremes. The average thyroid enlargement[7] was therefore 4.1–4.2 times the normal weight of the gland, while the extremes of enlargement were 2.3–7.9 times the normal weight. Based on these findings, it is apparent that Diet GP is markedly goitrogenic and is, therefore, a suitable diet for use in studies on goiter in the rat.

The largest gland removed actually weighed 117 mg. while, on the basis of 100 gm. body weight, the largest gland weighed 126.0 mg. When compared with the average normal thyroid weight, this latter gland weight would represent an enlargement of approximately 10 times.

The average thyroid weight of 20 stock rats at the beginning of the 35 day experimental period was 16.4 ± .32 mg. per 100 gm. body weight, while

[6] Thirteen stock rats sent to us through the courtesy of Dr. C. N. Frey of the Fleischmann Laboratories, New York City, yielded an average moist thyroid weight of 12.2 ± .27 mg. per 100 gm. body weight. The stock diet used was found by analysis in our laboratory to have an iodine content of 55γ per kilo.

[7] Enlarged thyroids were also found in 35 rats used in vitamin D assays and sent to us through the courtesy of Dr. Frey. These animals fed on the Steenbock rachitic diet yielded an average fresh thyroid weight of 44.7 ± 1.9 mg. per 100 gm. body weight with 25.8 to 75.2 mg. as the extremes of enlargement.

at the end of the experimental period the value for rats on the same diet averaged 12.9 ± .25 mg. This decrease in thyroid weight per 100 gm. body weight is accounted for by the fact that both in the rat (29–32) and in the human (28, 31) the thyroid is larger (on the basis of body weight) earlier in life.

When compared with the values given by Donaldson (33) for the thyroid weight of normal rats of the same body weight as our animals, our values

TABLE III

THE INFLUENCE OF SEX ON THE WEIGHT OF THE THYROID GLAND IN NORMAL AND GOITROUS RATS

| Diet | Iodine Content of diet γ per kilo. | Number of rats | | | Fresh thyroid weight (in mg.) per 100 gm. body weight | | Significance ratio |
|---|---|---|---|---|---|---|---|
| | | Total | Males | Females | Males | Females | |
| Age = 59–66 days | | | | | | | |
| GP | 15 | 193 | 102 | 91 | 52.0 ± 1.4 (24.1–114.0) | 56.9 ± 1.9 (25.0–126.0) | 2.0 Not significant |
| GPI | 400 | 24 | 13 | 11 | 12.4 ± .20 (10.8–14.7) | 12.8 ± .33 (10.5–16.0) | 1.0 Not significant |
| Stock | 47–72 | 39 | 21 | 18 | 12.9 ± .39 (9.0–18.6) | 12.9 ± .30 (10.0–17.6) | 0.0 Not significant |
| Age = 155–159 days | | | | | | | |
| Stock | 47–72 | 20 | 10 | 10 | 9.6 ± .24 (8.5–14.5) | 10.9 ± .23 (9.3–12.3) | 3.9 Significant |

for the weight of the gland obtained on the stock ration are lower. Thus, Donaldson's values for rats weighing 60 and 178 grams are 21.3 and 16.6 mg. per 100 gm. body weight, respectively, while our values at these body weights are 16.4 and 12.9 mg. On the other hand, McCarrison (34) obtained values for stock rats at these body weights which are lower than ours, i.e., 10.3 and 8.3 mg. per 100 gm. body weight. However, it is to be pointed out that our 60 and 178 gm. rats averaged only 27 and 62 days of age, respectively, whereas McCarrison's (35) animals at these body weights were 40 and 130 days of age. The smaller thyroid weights obtained by McCarrison may possibly be attributed therefore either to the poor growth of his rats, so that his animals in attaining these body weights had access

to the stock food for a longer period of time than our animals and hence were able to ingest more iodine, or to differences in the iodine content of the stock rations.

Figures 2 and 3 serve to show the differences in the size of thyroids obtained in our rats fed Diets GP and GPI.

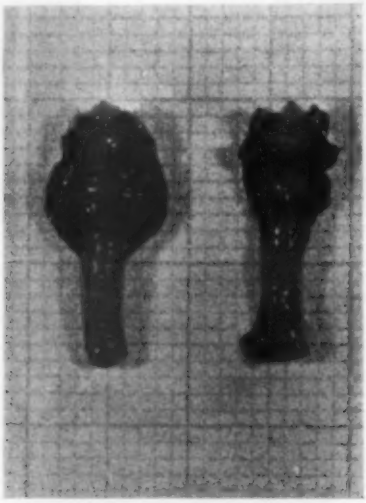
Rat 551 Diet GP | Rat 554 Stock Diet | Rat 553 Diet GPI

FIG. 1.—Showing the histological structure of the thyroid gland in three rats (litter mates) receiving various diets for 35 days. The goitrogenic diet (GP) contained 15γ iodine per kilo. of diet; Diet GPI representing the goitrogenic ration containing added iodine, had an iodine content of 400γ per kilo., while the stock ration contained 47–72γ of iodine per kilo. Note the hyperplasia and lack of iodine-containing colloid in Rat 551 in contrast to the normal cell structure and presence of colloid in Rats 553 and 554.

(d) *Comparison of the dry thyroid weights.*—Table II also demonstrates that the thyroid enlargement obtained on Diet GP is primarily an actual gain in tissue weight and not merely an accumulation of fluid. Thus, the

dried goitrous glands weighed 9.9 mg. per 100 gm. body weight while the stock diet and Diet GPI yielded glands weighing 3.0 mg. and 3.5 mg. respectively. On a dry basis, the enlargement was therefore 3.3 and 2.8 times.

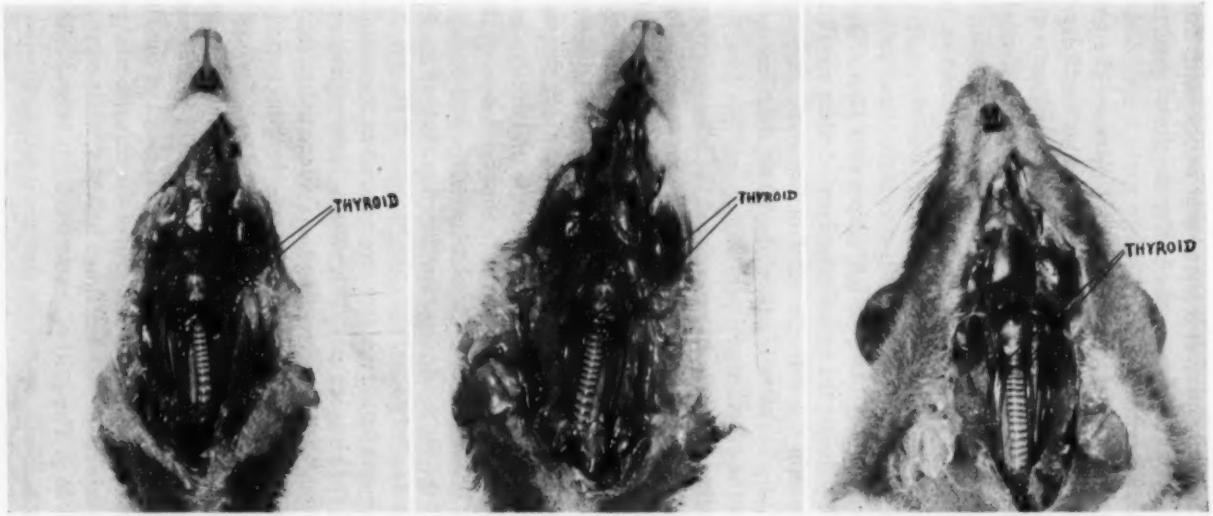
(e) *Comparison of the dry matter content of the glands.*—The goitrous glands contained only 19.0 per cent dry matter in contrast to 23.3 per cent for glands of rats on the stock ration and 28.0 per cent for glands of animals consuming Diet GPI, indicating that in the process of enlargement there is



Rat 551 Diet GP | Rat 553 Diet GPI

FIG. 2.—Showing the influence of iodine in the prevention of goiter. The photograph shows the glands attached to the excised tracheae. Rat 551 received the goitrogenic Diet GP while Rat 553 was fed Diet GPI for a period of 35 days. The actual fresh thyroid weights were 61.2 and 13.5 mg., respectively, while the fresh gland weights per 100 gm. body weight were 52.8 and 13.5 mg., respectively. The two rats were litter mates.

a definite accumulation of fluid. From this finding and the results just described relative to the extent of enlargement, it can be concluded that the production of goiter on Diet GP involves essentially a gain in tissue weight with the moisture content of the gland making a smaller but definite contribution. Oswald (36) also reported a higher water content in hyperplastic glands as compared with normal thyroids. By calculation from Marine's data (37) on the dog, the same finding is established.




Rat 550 Diet GP | Rat 551 Diet GP | Rat 545 Diet GPI

FIG. 3.—Showing the head and neck area with both normal and goitrous glands *in situ*. Rats 550 and 551 were fed the goitrogenic ration GP while Rat 545 was fed Diet GPI for a period of 35 days. Note the distention of the blood vessels leading to the thyroid in Rat 551. The actual fresh thyroid weights for Rats 550, 551 and 545 were 56.6, 61.2, and 11.7 mg., respectively, while the fresh gland weights per 100 gm. body weight were 57.8, 52.8 and 11.7 mg., respectively.

This finding that the goitrous glands were lower in dry matter content was confirmed later in our studies on the iodine requirement of the rat to be described in the following paper.[8]

(f) *Comparison of the iodine concentration in the glands.*—The goitrous glands were lowest in iodine concentration, i.e., 0.0083 per cent (dry basis) or 0.0016 per cent (fresh basis). These glands contained only approximately 1/8 as much iodine (dry basis) or about 1/9 as much (fresh basis) as glands from rats fed the stock diet for 35 days. When compared with glands from animals fed the iodine-containing Diet GPI, these values become approximately 1/32 and 1/47, respectively.

At the start of the experimental period, the glands contained an average of 0.0428 per cent iodine (dry basis) or 0.0110 per cent (fresh basis). In the production of goiter during the experimental period there was therefore a considerable decrease in the iodine content of the gland, the thyroids containing only 1/5 (dry basis) or 1/7 (fresh basis) as much iodine at the end as at the start of the experimental period.

In the human, Marine (28), who has correlated the histological appearance of the thyroid with its iodine content, reports an average iodine content of 0.0328 per cent dry basis (range, 0.0118–0.0584 per cent) for "marked glandular hyperplasia." In the dog, Marine and Williams (37) found an average iodine content of 0.0114 per cent (dry basis) for glands with marked hyperplasia. These goitrous glands in the dog weighed approximately 4.2 times the normal, which represents practically the same extent of glandular enlargement that we are dealing with here in the rat. Our average value of 0.0083 per cent (dry basis) for the iodine content of the goitrous gland of the rat would indicate therefore that we are dealing with a well-advanced stage of goiter.

McCarrison (35) reports 0.0182 per cent (fresh basis) as the mean iodine content of the thyroid of his stock rats. This value is higher than our value of 0.0147 per cent (fresh basis) representing the average iodine content of the thyroid of our stock rats at the end of the experimental period.

(g) *Comparison of the total iodine content of each gland.*—A study of Table II reveals that the goitrous glands contained the least amount of iodine per gland, while Diet GPI yielded thyroids with the highest iodine content.

The actual average total amount of iodine in each gland at the start of the experimental period was 1.11 $\gamma$. At the end of the experimental period, in rats on the goitrogenic ration this had fallen to 0.88 $\gamma$, while on the stock diet it increased to 3.36 $\gamma$. The goitrous rats ingested 0.14 $\gamma$ of iodine per

[8] *This Journal*, p. 347.

day or a total of 4.9 γ for the entire experimental period. It is evident therefore that this intake of iodine is insufficient to provide for the normal increase in iodine content of the thyroid gland coincident with advancing age and body weight (32, 35), with the result that thyroid enlargement or goiter develops as a compensatory process.

(h) *Comparison of the iodine intakes.*—The goiter-producing diet which was lowest in iodine content (15 γ per kilo.) afforded the lowest intake of iodine, i.e., 0.14 γ per rat per day. The stock ration which, as indicated in Table I, varied in iodine content from 47 to 72 γ per kilo. due to the purchase of new batches of ingredients from time to time, furnished an average daily iodine intake of 0.74 γ, approximately 5.5 times the amount provided by Diet GP. Diet GPI containing 400 γ of iodine per kilo. yielded an average daily intake of 3.72 γ, approximately 27 times the amount furnished by the goitrogenic ration.

(i) *Influence of Sex on Thyroid Weight.*—Since it is generally recognized that goiter in the human (28, 31) occurs with greater frequency in the female than in the male and that female glands are larger than male glands, we analyzed the data included in Tables I and II in order to determine whether there were any sex differences in the gland weight of normal and goitrous rats. Table III, which serves to show the results obtained, reveals the fact that among neither normal nor goitrous glands of young rats was significant sex difference in the weight of the glands apparent. In this connection, it is to be emphasized that these rats were only 59 to 66 days of age at autopsy and hence had not reached full sexual maturity. The influence of sex, therefore, would hardly have had an opportunity to assert itself. In order to determine whether older and sexually mature rats would show a sex difference in thyroid weight, three litters of rats containing 10 males and 10 females raised on the stock ration were killed at 155 to 159 days of age. The males averaged 313 gm. in body weight and the females 207 gm. The average thyroid weights are given in Table III. When the averages, $9.6 \pm .24$ and $10.9 \pm .23$ mg. per 100 gm. body weight were treated statistically, the difference of 1.3 mg. in gland weight was found to be significant. This finding indicates that in sexually mature normal females fed our stock ration the gland is slightly though significantly heavier than in male rats of the same age.

## Discussion

In 1928, Marine (38) wrote:

"As a result of the numerous studies on the relation of iodine to the

thyroid gland our present views regarding the cause of goiter assume that it is a compensatory or work hypertrophy of the thyroid depending upon a relative or absolute deficiency of iodine. This deficiency of iodine may be due to: 1.—Factors which bring about an abnormally low intake of iodine. 2.—Factors which interfere with the absorption or utilization of an otherwise adequate intake. 3.—Factors which increase the needs of the body for the iodine-containing hormone." Accordingly, several possible explanations may be offered concerning the manner in which the goitrogenic Diet GP operates to produce thyroid enlargement and hyperplasia in the rat. This diet, as previously stated, is low in iodine content, yielding an average daily iodine intake of only 0.14 $\gamma$. Goiter could develop on this diet in several possible ways: 1.—the amount of iodine furnished in the ration may be insufficient, when absorbed into the blood stream, to provide for normal thyroid function; 2.— the iodine may be present in the diet in a form unabsorbable or poorly absorbed in the intestinal tract; 3.—the iodine, if absorbed into the blood stream, may not be in a form utilizable by the thyroid gland to elaborate thyroxin; 4.—a combination of an inadequate iodine intake and poor absorption or utilization of this iodine, or 5.—the nature of the diet itself or some factor in the diet may bring about an increased demand for iodine. Webster and co-workers (39) and McCarrison (40) have shown that the ingestion of cabbage or certain other vegetables by rabbits creates an increased demand for iodine, resulting in goiter. In a review of the literature, Marine (41) points out that meat, liver and fat have been shown to be capable of producing thyroid hyperplasia in various animals, while Stott and co-workers (42) report that an excess of calcium in the drinking water is a causative agent in the production of goiter in human beings in India.

That the rat is able to utilize enough iodine from the goitrogenic ration to maintain a certain extent or degree of glandular enlargement is borne out in an experiment in which it was found that rats (litter mates) fed the diet for a period of either 35 or 124 days yielded essentially the same degree of thyroid enlargement. Further, in a preliminary basal metabolism experiment, it was found that four rats fed the goitrogenic diet for a period of eight weeks showed a basal metabolic rate of approximately 23 per cent below normal. When the animals were continued on the same ration for seven weeks longer, no further significant lowering of the metabolic rate occurred. These facts would seem to indicate that the goiter-producing ration GP is unable to furnish the rat with a supply of utilizable iodine sufficient to provide for normal thyroid function or the production of

adequate amounts of thyroid hormone, but does yield an amount of available iodine which limits the extent of thyroid enlargement and hyperplasia and then apparently maintains a subnormal functioning of the gland. For these reasons, we are making further efforts to devise a goitrogenic diet containing an even lower content of iodine so that even more severe goiter may be obtained.

In order to throw some light on the exact manner in which goiter develops in the rat, it would be necessary to follow carefully the paths of excretion of the iodine contained in the goitrogenic ration by carrying out metabolism studies. Such a procedure would simultaneously determine the availability of the small amount of iodine in this diet. By including, in such a study, rats fed on our stock ration and on Diet GPI representing a ration containing an adequate amount of iodine, further data concerning the metabolic behavior of iodine could be obtained. It is hoped to carry out such studies in a future investigation.

The Steenbock diet produces rickets in the rat by virtue of an abnormally high Ca:P ratio, i.e., approximately 4.3:1. This necessitates the inclusion of $CaCO_3$ in the ration so that a high calcium content results. Stott and co-workers (42) believe that goiter in India is directly related to the high calcium content of the drinking water, while Hellwig (12) reports the production of goiter in the rat by means of $CaCl_2$. On the other hand, Simpson (43) found that the addition of lime to the diet of rabbits did not cause thyroid enlargement. For these reasons, we became interested in ascertaining whether the high calcium content or the abnormal Ca:P ratio of our goitrogenic diet may not be the cause of goiter in our rats or be one of the factors that bring it about. Preliminary results obtained on this phase of our investigations indicate that neither the high calcium content nor the abnormal Ca:P ratio are responsible for the goitrogenic activity of the diet. Further, by replacing the 3 per cent $CaCO_3$ and 1 per cent NaCl in the goitrogenic ration with 4 per cent of Osborne-Mendel inorganic salt mixture (without KI) having a Ca:P ratio similar to that of cow's milk, we were still able to produce goiter. We are continuing our studies along this line, particularly because Baumann, Kurland, and Metzger (44) have recently reported that there is a definite retention of calcium, magnesium, and phosphorus in goiter produced in rabbits by cabbage feeding. When iodine is administered to cure such goitrous animals, there is a restoration of the balance of these elements by increasing the amounts excreted. As a result of this finding, Baumann and co-workers state that they "have reason for inferring that calcium retention is directly associated with the

development of simple goiter." Aub and his co-workers (45) report that the calcium excretion in myxedema is markedly diminished below that found in normal individuals.

The fact that the well-known Steenbock ration produces goiter both in the presence and in the absence of vitamin D has some bearing on the technic employed in the assay of foods for vitamin D. In such an assay, rats weighing 60 grams are placed on the Steenbock diet for a preliminary period of 18–21 days in order to produce rickets and are then fed for an additional ten-day period during which time the diet is supplemented with the food substance under test. Our results reported here show conclusively that in such a procedure, goiter as well as rickets is present at the end of the preliminary period, while in the curative period goiter may still be present while the rickets is being healed. If the vitamin D supplement happens to be cod liver oil, a rich source of iodine[9] then it is possible that both the rickets and the goiter may be cured at the same time. What effect the presence of goiter has on the prevention or cure of rickets we cannot say at this time. As yet we have no evidence to indicate that the presence of an adequate amount of iodine throughout such an assay would have any effect on calcification of bone and hence on the determination of the vitamin D potency of a food, but it is well to point out to investigators who are using the Steenbock diet in vitamin D assays, that the two dietary deficiency diseases are simultaneously present. Since in all types of biological assays the aim is to have only one dietary deficiency present, we therefore recommend that iodine be added to the drinking water of the rats throughout the vitamin test so that only rickets will be present. Although we hesitate to suggest at this time that iodine plays a rôle in bone metabolism, it is possible that the better clinical results (46, 47) obtained with cod liver oil as compared with viosterol both fed at the same number of vitamin D units may be due to the presence of iodine in cod liver oil and its absence in viosterol. Recently, Russell (48) in experiments on chickens has shown that the addition of vitamin A failed to increase the effectiveness of irradiated ergosterol sufficiently to make it equivalent in antirachitic potency to cod liver oil when the same number of vitamin D units of each substance was fed. This latter finding makes it appear that some factor other than vitamin A is responsible for the difference in antirachitic potency.

The question might be raised as to whether or not our stock ration—a

[9] Analyses in our laboratory show a very high iodine content for cod liver oil, i.e., approximately 10,000$\gamma$ per kilo.

modification of the well-known Sherman breeding diet—contains an adequate amount of iodine. The thyroids from rats fed our stock ration did not have the pale pink color of the glands of rats fed Diet GPI, but were slightly red, despite the fact that the gland weights per 100 gm. body weights were practically the same on each diet; histologically, they were slightly below normal,[10] while the glandular iodine concentration—0.0634 per cent (dry basis)—was below the value of 0.1 per cent (dry basis) or 1 mg. iodine per gram of dried gland which Marine (41) states is the minimum amount necessary for the maintenance of normal gland structure. It is, however, possible that the ingestion of 0.74 $\gamma$ of iodine daily by our rats on the stock ration is adequate since good reproduction, lactation, and growth have been obtained in our breeding colony for many generations. That good growth also resulted in our experimental animals on this ration is evidenced by the fact that the rats attained an average body weight of 62 gm. at 27 days of age and an average body weight of 178 gm. at the age of 62 days. This rate of growth compares quite favorably with that reported by other workers using different stock diets.[11] Whether even better growth and reproduction would result if our stock diet were supplemented with iodine is not known. We hope to investigate this aspect of iodine metabolism soon.

The use of Diet GP in goiter studies would seem to possess several advantages over the cabbage feeding technic proposed by Webster (39). Diet GP produces in the rat an average thyroid enlargement of 4.1–4.2 times the normal gland weight in a period of 35 days. All five constituents of the diet are present as a composite mixture of dry ingredients which are inexpensive and are easily obtained in large enough quantities to last over an extended period of time. On the other hand, the cabbage must be steamed and then fed in a definite quantity each day. Again, it is definitely known that the goitrogenic activity of cabbage varies with the season of the year, and with the locality. This fact would make it necessary to preserve large quantities of the same lot of fresh cabbage for an experiment lasting for a long period of time. The fact that rats can be bred in large numbers on a standardized

[10] Mendel and Vickery (49) report that "rats raised in our laboratory on diets without any additions of iodine other than that present in the natural foods and drinking water used showed somewhat defective thyroids." Likewise, Thompson (21) reports abnormal thyroids in rats on a breeding ration.

[11] A sample of the stock diet used at the Fleischmann Laboratories was sent to us by Dr. Frey. It was found to contain 55$\gamma$ per kilo. of iodine. This value compares quite well with the value of 47–72$\gamma$ per kilo. for our stock ration.

stock ration insures a continuous supply of uniform animals and hence uniform results in experimental work.

## Summary

A dietary technic suitable for goiter studies employing the rat as the experimental animal has been described. The goiter-producing diet used is low in iodine, having an average content of 15 $\gamma$ per kilo., and yielding an average daily intake of 0.14 $\gamma$ per rat.

A total of 193 rats were fed the goitrogenic ration for a period of 35 days. The following effects on the thyroid gland were observed: 1.—dark red color; 2.—marked hyperplasia with little or no iodine-containing colloid; 3.—average fresh weight of $53.2 \pm .92$ mg. per 100 gm. body weight (range, 24.1–126.0 mg.), representing an average enlargement of 4.1–4.2 times the normal gland weight with 2.3–7.9 as the extremes of enlargement; 4.—a low dry matter content—19.0 per cent; 5.—a low concentration of iodine in the gland, i.e., 0.0083 per cent (dry basis) or 0.0016 per cent (fresh basis); and 6.—a low total iodine content per gland of 0.88 $\gamma$.

The production of goiter on this ration involves essentially a gain in tissue weight of the gland with the moisture content of the gland making a smaller but definite contribution.

A total of 24 rats were fed the goitrogenic ration plus added KI to furnish approximately 3.72 $\gamma$ of iodine per rat daily, yielding the following results on the thyroid gland at the end of 35 days: 1.—normal pale pink color; 2.—normal histological structure with an abundance of colloid; 3.—an average fresh weight of $12.6 \pm .19$ mg. per 100 gm. of body weight (range, 10.5–16.0 mg.); 4.—a dry matter content of 28.0 per cent; 5.—an iodine concentration of 0.2671 per cent (dry basis) or 0.0745 per cent (fresh basis) and 6.—a total iodine content per gland of 10.28 $\gamma$.

Comparable results for the thyroid are also given for 39 rats fed on a stock ration containing 47–72 $\gamma$ of iodine per kilo.

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# STUDIES ON THE RELATION OF DIET TO GOITER*

## II. THE IODINE REQUIREMENT OF THE RAT†

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AFTER we had developed a suitable dietary technic (1) for goiter studies using the rat as the experimental animal and had also gained some knowledge concerning the weight, iodine content, dry matter content, etc., of the normal and the goitrous rat thyroid, we next undertook to determine the iodine requirement of the rat. We are interested in this phase of our goiter research for two reasons; first, we feel that by determining the minimum iodine requirement of the rat we may be able to throw some light, indirectly, on the question of what constitutes the human requirement for iodine, a subject which lacks much needed direct experimental evidence; and second, we are anxious, in later studies, to compare the availability of various forms or sources of iodine, such as plant and animal iodine, di-iodotyrosine, thyroxin, iodized salt, potassium iodide, etc. In order to attack this latter problem adequately, it was considered necessary to feed the various sources of iodine in amounts *at* or *near* the minimum iodine requirement of the rat so that small differences in availability or utilizability, if present, may appear. This same principle of making comparisons at or near the minimum requirement is used in many studies in nutrition where comparisons of different sources of various dietary essentials are to be made, i.e., vitamins, inorganic elements, proteins, etc. In practically all earlier experimental feeding of iodine compounds to animals, the amounts used have been enormously greater than the physiological requirement (2–9).

Accordingly, we have made a study of the iodine requirement of the rat and have carried out two groups of experiments involving a total of 100 rats.

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## Experimental

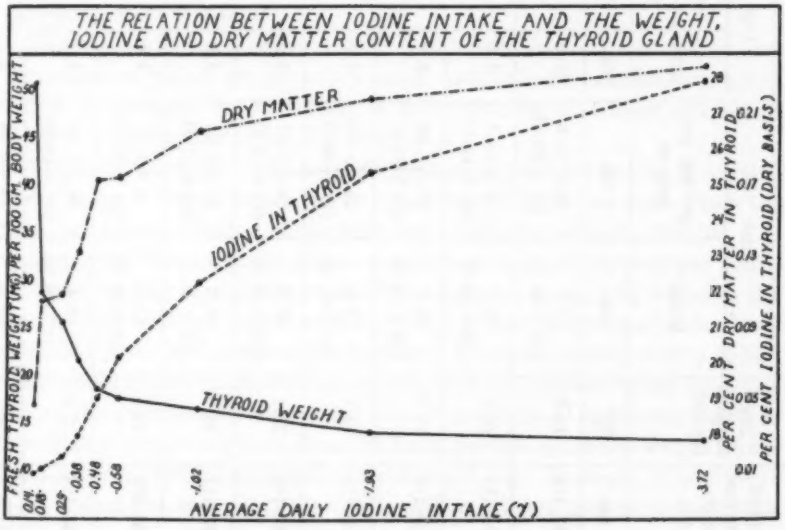
In the development of a dietary technic for the study of goiter in the rat (1), we found that the goiter-producing ration (Diet GP) furnished an average daily iodine intake of 0.14 γ. It was evident, therefore, that a daily intake of iodine greater than this amount was necessary to prevent thyroid enlargement *on this diet.* Accordingly, in our first group of experiments we prepared a series of five diets containing different amounts of iodine, by evaporating various amounts of KI on Diet GP. These five rations—Diets GP, GPE, GPF, GPG and GPH—ranged in iodine content from 15 γ per kilo. for Diet GP to 400 γ per kilo. for Diet GPH (See Table I), yielding average daily iodine intakes per rat of 0.14, 0.59, 1.02, 1.93 and 3.72 γ, respectively. These diets were fed to groups of ten rats each with the exception of Diet GP which was fed to a group of 20 rats. Ten litters of rats raised on our stock ration (1) having an iodine content of 47–72 γ per kilo. were employed. The animals weighed approximately 60 gm. and were distributed so that litter mates were represented in each of the five groups of rats. The sexes were also divided evenly as far as possible. The experimental period lasted 35 days, at the end of which time the rats were killed and the thyroids removed. The fresh thyroid weight was immediately determined after which all the glands of rats on the same diet were pooled and dried to constant weight at a temperature of 80°C. The average iodine concentration in the glands was determined by a modification of the micro-McClendon method (10).

Based on our findings in this first group of experiments, it was evident that a study of the influence of iodine intakes lower than 0.59 γ daily would yield important information. Accordingly, we carried out a second group of experiments in which four additional diets—Diets GPA, GPB, GPC and GPD—were fed. These rations ranged in iodine content from 20–51 γ per kilo., yielding intakes of 0.18, 0.29, 0.38, and 0.48 γ of iodine per day, respectively (see Table I). In this experiment, 40 rats, evenly divided as to litter and sex, were used.

The results of these two groups of experiments are given in Table I and in Chart 1. From a study of these data, it is at once evident that as the iodine intake increased, there was a corresponding decrease in the thyroid weight, while the iodine concentration in the gland, the total iodine content of each gland, and the dry matter content of the gland increased. In other words, as goiter develops the moisture content of the thyroid increases, while the iodine concentration in the gland decreases. Apparently the so-called "thyroid colloid" which fills the acini of normal glands has a

lower water-holding power than the cytoplasm of basement cells, which in goitrous glands are found to be much enlarged. In our previous study (1), we reported that goitrous glands were dark red, in contrast to normal thyroids which were pale pink in color. In the present experiment, we observed that as the daily iodine intake increased with the feeding of the various rations, there was a simultaneous lessening in the intensity of the red color of the gland until the normal pale pink color was reached. The increased

Chart 1.


THE RELATION BETWEEN IODINE INTAKE AND THE WEIGHT, IODINE AND DRY MATTER CONTENT OF THE THYROID GLAND
DRY MATTER
IODINE IN THYROID
THYROID WEIGHT
FRESH THYROID WEIGHT (MG.) PER 100 GM. BODY WEIGHT
PER CENT DRY MATTER IN THYROID
PER CENT IODINE IN THYROID (DRY BASIS)
AVERAGE DAILY IODINE INTAKE (γ)
0.36
0.48
0.58
1.02
1.83
3.72

blood supply of goitrous glands may be a factor in the observed increase in water content.

From the results of these two groups of experiments, the following facts are revealed:

An inverse relationship exists between; 1.—the iodine intake and the weight of the thyroid gland; 2.—the iodine concentration in the gland and the weight of the thyroid; 3.—the dry matter content and the weight of the thyroid; 4.—the iodine intake and the intensity of the color of the gland.

A direct relationship exists between; 1.—the iodine intake and the iodine content of the thyroid; 2.—the iodine intake and the dry matter content

TABLE I

A SUMMARY OF DATA ON THE IODINE REQUIREMENT OF THE RAT, SHOWING THE RELATON BETWEEN IODINE INTAKE AND THE WEIGHT, IODINE AND DRY MATTER CONTENT OF THE THYROID GLAND

(All rats started at approximately 60 g. body weight* and fed experimental diets for 35 days)

| Diet | Iodine content of diet | Iodine intake | | No. of rats | Initial and final body wts. | Dry matter in thyroid | Average thyroid weight | | | | Iodine conc. in gland | | Total iodine in each gland | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Fresh | | Dry | | | | | |
| | | Av. daily | Av. total | | | | Actual | per 100 gm. body wt. | Actual | per 100 gm. body wt. | Dry basis | Fresh basis | Actual | per 100 gm. body wt. |
| | γ/kilo. | γ† | γ | | gm. | % | mg. | mg. | mg. | mg. | % | % | γ | γ |
| GP | 15 | 0.14 | 4.9 | 20 | 62–114 | 18.8 | 57.7 (32.7–97.7) | 50.6±2.6 (27.7–84.4) | 10.8 | 9.5 | .0070 | .0013 | 0.76 | 0.67 |
| GPA | 20 | 0.18 | 6.4 | 10 | 63–115 | 21.7 | 32.9 (20.8–44.9) | 28.6±1.3 (21.5–41.3) | 7.1 | 6.2 | .0102 | .0022 | 0.73 | 0.63 |
| GPB | 31 | 0.29 | 10.3 | 10 | 60–114 | 21.9 | 29.1 (24.7–34.9) | 25.5±.80 (19.9–31.2) | 6.4 | 5.6 | .0165 | .0036 | 1.05 | 0.92 |
| GPC | 41 | 0.38 | 13.3 | 10 | 60–111 | 23.1 | 23.8 (16.8–33.6) | 21.4±.84 (16.2–26.0) | 5.5 | 4.9 | .0282 | .0065 | 1.55 | 1.39 |
| GPD | 51 | 0.48 | 16.8 | 10 | 61–114 | 25.1 | 20.9 (15.3–26.8) | 18.4±.61 (14.9–24.4) | 5.3 | 4.6 | .0494 | .0124 | 2.53 | 2.28 |
| GPE | 63 | 0.59 | 20.7 | 10 | 61–115 | 25.2 | 19.9 (17.0–24.5) | 17.5±.41 (14.8–21.5) | 5.0 | 4.4 | .0729 | .0184 | 3.57 | 3.21 |
| GPF | 110 | 1.02 | 35.7 | 10 | 63–106 | 26.5 | 17.3 (14.0–20.9) | 16.4±.49 (13.5–20.5) | 4.6 | 4.3 | .1150 | .0305 | 5.27 | 4.94 |
| GPG | 207 | 1.93 | 67.6 | 10 | 63–110 | 27.4 | 15.4 (11.7–19.5) | 14.0±.44 (11.9–18.8) | 4.2 | 3.8 | .1765 | .0484 | 7.43 | 6.71 |
| GPH | 400 | 3.72 | 130.2 | 10 | 64–114 | 28.3 | 14.9 (11.4–17.7) | 13.1±.36 (10.5–16.0) | 4.2 | 3.7 | .2275 | .0644 | 9.60 | 8.42 |

* Average age = 27 days.

† 1 mg. = 1000 γ.

of the gland; 3.—the iodine concentration in the gland and the dry matter content.

A further study of the chart reveals the fact that the area represented by iodine intakes ranging from 0.14–0.59γ is a very sensitive or critical one, in that small increments or decrements in iodine intake in this area result in marked abrupt changes in the weight, iodine concentration, and dry matter content of the thyroid. On the other hand, for iodine intakes between 0.59–3.72 γ, the effect on the gland is much less marked, particularly for the higher intakes in this range, indicating thereby a tendency of the thyroid to reach constant values.

The highest iodine intake—3.72 γ—yielded thyroids with an iodine concentration of 0.2275 per cent (dry basis) and an iodine content per gland of 9.60 γ. It would be interesting to determine the maximum amount of iodine the rat thyroid is capable of storing.

The point at which the actual physiological need for iodine is fulfilled and storage of iodine begins can be approximated from our data. From the study of a large number of thyroids from different animals, Marine (11) has reported that when the iodine concentration in the thyroid was below 0.1 per cent (dry basis), hyperplastic changes were regularly found in the gland. If we consider, therefore, 0.1 per cent as the minimum effective level of iodine, then by interpolation in Chart I, approximately 0.90 γ iodine daily would appear to be the minimum iodine requirement of the rat when fed on the goiter-producing ration GP. This intake of iodine corresponds to a fresh thyroid weight of about 16.5 mg. per 100 gm. body weight and a dry matter content of approximately 26.0 per cent. However, it is evident from a study of our data, that daily iodine intakes greater than 0.90 γ yielded glands lighter in weight than 16.5 mg. per 100 gm. body weight and containing a higher concentration of iodine and a higher dry matter content. It would seem, therefore, that a better criterion for the minimum iodine requirement would be the *smallest amount of iodine necessary to prevent any significant glandular enlargement.* In our experiments, Diets GPF and GPG furnishing 1.02 and 1.93 γ of iodine daily yielded thyroid weights of 14.0 ± .44 and 13.1 ± .36 mg. per 100 gm. body weight, respectively. When statistically treated, the difference of 0.9 mg. in thyroid weights in these two groups was found to be not significant. Using the above criterion, therefore, the minimum daily iodine requirement of the rat when fed the goitrogenic ration would be approximately 1–2 γ iodine, this intake of iodine corresponding to a glandular iodine concentration of approximately 0.11 to 0.18 per cent (dry basis).

* Average age = 27 days.

Obviously, more accurate data, particularly histological findings, are needed in order to locate more definitely the minimum effective level of iodine. We are continuing this phase of our research and are planning to correlate thyroid weight, iodine and dry matter content with histological findings at various intakes of iodine.

## Discussion

Views as to human iodine requirement have up to this time been based almost entirely on analysis of diets in goitrous and non-goitrous regions, or on the iodine elimination of individuals in such regions. Summarizing such available information, Cameron (12) states that the minimum quantity of iodine required has been estimated to amount only to between 35 and 70 $\gamma$ per day, while Orr and Leitch (9) state that the minimum daily requirement may be about 45 $\gamma$ for an adult male and 150 $\gamma$ for a child. If it is assumed that the average daily caloric intake is 50 calories for the rat and 3,000 calories for the average human, then a calculation based on the relative caloric intake gives a factor of 60. Taking the iodine requirement of the rat at 1–2 $\gamma$ per day, the human requirement would then amount to approximately 60–120 $\gamma$ per day, or if we take the value of 0.9 $\gamma$ daily for the rat as the minimum amount of iodine necessary to provide 0.1 per cent of iodine (dry basis) in the rat thyroid, this value would yield a corresponding value of approximately 54 $\gamma$ for the human. Such a method of comparison will doubtless be criticized, but is certainly more reliable than calculations based on relative body weight, as is sometimes done in toxicity studies. When the results of these calculations are compared with the estimates given above by Cameron and Orr and Leitch, the agreement is striking.

That very little is known regarding the iodine requirement of animals is evident from a recent review of the literature by Orr and Leitch (9). Thus, these investigators state: "Regarding the iodine requirements of animals we have no definite information. Nor can we deduce the requirement of farm animals from a knowledge of the amount required to prevent goiter, for as yet no standard and adequate dosage has been determined. The doses recommended by different workers vary widely and are probably all unnecessarily high." Until precise data are obtained for different species of animals *we suggest, therefore, that 20–40 $\gamma$ per 1,000 calories of the ration be considered as the minimum iodine requirement for farm animals.*

It is a common practice in most laboratories employing synthetic diets in experiments on rats to include 4 per cent of Osborne-Mendel inorganic salt mixture (13) in order to furnish all the essential mineral elements, in-

cluding iodine. This salt mixture contains 0.0025 per cent of iodine so that a daily intake of 10 grams of food yields 10 γ of iodine. Mendel and Vickery (14) raised the question as to whether this amount of iodine is sufficient for normal growth in the rat. They investigated the possible desirability of increasing the iodine content of this salt mixture and found no improvement in growth of rats when additional iodine (equivalent to 844 γ daily as KI) was added to a synthetic diet already containing 4 per cent of the salt mixture. Considering our own findings on the iodine requirement of the rat, it is evident that 10 γ of iodine daily provides a quantity of this element from five to ten times the minimum need and is therefore undoubtedly adequate for normal functioning of the thyroid gland.

The fact that the rat requires such a small amount of iodine may explain the inability of some investigators to produce goiter in the rat by diet. Thus, Jackson and P'an (15) who failed to produce goiter on a low-iodine synthetic ration report[1] that the iodine content of their ration approximated 1 γ per day. Likewise, Hellwig (16) who concluded from his experiments on rats that insufficiency of iodine is not the essential cause of goiter may have fed his animals enough iodine to prevent goiter, for he assumed, without analysis, that the iodine content of his experimental rations was low.

## Summary

The minimum iodine requirement of the rat was determined by feeding various groups of rats on a goitrogenic diet to which different amounts of iodine (as KI) were added. Using as a criterion *the smallest amount of iodine necessary to prevent any significant thyroid enlargement*, the minimum iodine requirement was found to be approximately 1–2 γ per rat per day. This intake of iodine yielded a concentration of 0.11–0.18 per cent iodine (dry basis) in the thyroid.

The daily intake of iodine necessary to yield a concentration of 0.1 per cent iodine (dry basis) in the thyroid was found to be approximately 0.9 γ.

The inclusion of 4 per cent Osborne-Mendel salt mixture in synthetic diets for experiments with rats provides from 5–10 times the minimum iodine requirement of the rat.

Using for comparison the results obtained in the rat, the minimum iodine requirement of the human was calculated to be approximately 60–120 γ per day.

[1] Personal communication.

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# THE EFFECTS OF IRRADIATED ERGOSTEROL ON THE METABOLISM OF NORMAL DOGS*

*By*

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IN THE course of experiments designed to elucidate the problem of the mechanism of action of irradiated ergosterol (Reed and Thacker, 10) it was noted that if normal animals were given large doses over some time, they invariably lost weight. This suggested the possibility of involvement of protein metabolism and, therefore, the further possibility of an influence on basal metabolism. These possibilities seemed the more reasonable in view of the known low metabolic rate of rachitic subjects (Seel, 11).

Consequently, the major portion of the investigation herein to be reported has to do with the effects of irradiated ergosterol on basal metabolism and the correlation of these effects with alterations in nitrogen metabolism, and with the mobilization and excretion of calcium and phosphorus.

The dogs were kept in metabolism cages during the period of observation. A standard diet of beef heart and puppy meal, ratio of 3:1 by weight, was used throughout, each new portion being analyzed for nitrogen, calcium, and phosphorus. The weight of the food consumed by each dog was recorded carefully. Any residue after feeding was preserved and weighed. The detailed data for food consumption were not included in the graphs because this would only complicate the presentation and would not add materially to the discussion of food consumption included in the text. The food nitrogen was determined by the Kjeldahl method as described by Hawk and Bergeim (5), using 2 grams of dried food. For the inorganic constituents 10 grams of the dried food were placed in a vitrosil crucible and heated at 800°C. until only a white ash remained. This was then dissolved in nitric acid and evaporated to dryness. Next, the material was dissolved in 6 N hydrochloric acid, filtered and diluted to 100 cc. From this

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filtrate, calcium and phosphorus were determined by the methods described for urine.

Urine was collected daily at 9:30 a.m. and analyzed for total nitrogen by the Kjeldahl method, urea N by the Folin and Youngburg method, creatinine by the Folin method (3), calcium by the Shohl and Pedley method (12), and inorganic phosphorus by the method of Fiske and Subarrow (2). Toluol was placed in the urine receptacles as a preservative.

The metabolism cages were cleaned three times each week and the feces saved. The feces collection from each cage for each week was dried and thoroughly mixed and three samples taken, ranging from 1.0 to 1.5 grams. Each sample was transferred to a Kjeldahl flask with 25 cc. of concentrated sulphuric acid, 25 cc. of water, and 5 grams potassium sulfate, and allowed to stand over night. The further procedure was the same as for urine. Ten gram samples of feces were weighed into large vitrosil crucibles and ashed at 800°C., after which the rest of the procedure was as described above for calcium and phosphorus.

The animals were bled before feeding or injecting. About 10–12 cc. of blood were drawn directly from the heart by needle and syringe, using heparin as an anticlotting agent. Plasma calcium was determined by Clark and Collip's modification of the Kramer-Tinsdall method. Inorganic phosphorus was determined by the Fiske and Subarrow method (2). Kerr's procedure (7) for potassium was used.

At the termination of each experiment an autopsy was performed and tissues saved for chemical and microscopic studies. The tissues were weighed immediately, then dried at 105°C. for three days and again weighed into a vitrosil crucible and ashed at 800°C. The ash was dissolved in nitric acid and evaporated to dryness. The material was then dissolved in 10 cc. of 20 per cent trichloroacetic acid, and diluted to 25 cc. in a volumetric flask. Portions of this filtrate were used for calcium determination. The phosphorus was determined as in blood.

The basal metabolic rate was determined on trained dogs by the Sanborn-Benedict machine, as described by Kunde and Steinhaus (9). All the necessary precautions as to training and technic were observed, and food consumption standardized during the period of preliminary observation.

Calculation of surface area was made by the Meeh formula. While this formula is satisfactory for comparative purposes with subjects of constant weight, it is apparent that the loss of weight introduces a complication for which no satisfactory solution has yet been devised. In some of the figures are presented two graphs of metabolic rate, one based on the

daily weight fluctuations, the other on the original average weight over a period of days. In every instance the second graph shows a lower metabolic rate. The two graphs do not materially diverge, however, until after there has been considerable loss of weight. Consequently, the conclusions as to the early effects on the metabolic rate are not invalidated. In later stages the true metabolic rate will obviously lie somewhere between the two calculations.

Another possible source of error in these calculations lies in the fact that the figure 4.83 was used throughout as the calorific value of oxygen. This assumes a respiratory quotient of approximately 0.83. We believe that the quotient does not remain constant. This belief is grounded on the fact that the fat stores are completely exhausted after a time. In fact it seems probable now that most, if not all, of the early weight loss is to be ascribed to consumption of body fat. In that case the calorific value of oxygen would be expressed by a much lower figure and consequently the entire curve of metabolic rate would be appreciably lower than represented, except possibly in the earlier stages, before weight loss is manifest.

It has been shown that when ergosterol is administered by mouth there is likely to be some reduction to coprosterol in the intestinal tract, and therefore some loss in potency. Since the proportionate loss necessarily varies in different subjects and under variable conditions, it was deemed necessary first to adopt a method of administration that would insure constant potency of the material applied to the effector mechanism, whatever it may be. Furthermore Klein and Russel (8) have shown that it is possible to recover from the feces 26 to 43 per cent of irradiated ergosterol fed by mouth. Reed and Thacker (10) have demonstrated that the material in oil can be quantitatively administered by intravenous injections. This method was first applied in this laboratory in February, 1929. The question has been raised, frequently, as to the danger of oil emboli. In more than 1,000 individual injections, in this laboratory, of ergosterol in oil or of corn oil alone, in amounts up to 15 cc., to both dogs and human subjects, there have been no indications of emboli. Therefore it is believed that this is a safe and reliable method. Taylor (13) and his co-workers also report successful intravenous administration to animals.

To our knowledge, there have appeared in the literature only three reports of experiments on the effect of irradiated ergosterol on the metabolic rate. Seel (11) reported that this substance restored to normal the lowered metabolic rate of rachitic rats. Thöenes (14) found that there was an increased gaseous exchange in normal subjects that bore no quantita-

tive relation to dosage and that nitrogen exchange was increased. Handowsky (4) believed that ergosterol is a metabolic stimulant similar to saponin.

In a series of experiments on nitrogen balance in one human subject and five dogs, two of which were studied during two relatively long periods, it was impossible to demonstrate any constant variation in nitrogen metabolism. The total nitrogen excretion and balance, and the urea and creatinine excretion were subject only to such variations as might occur normally.

### Basal Metabolism

Each dog was subjected to a long period of training and the average for the period after a fairly stable value had been established was taken as the basal rate. This training period was much longer than is shown in the charts, since the starting point on each illustrative case is taken as the day on which the animal was placed under strictly standardized conditions. For this reason in the foreperiod, as shown in the illustrations, the average of the rate is sometimes below the basal figure. This does not, however, invalidate the comparative results.

In Figure 1 (Dog No. 17) the basal rate was 800 cal./sq.m./24 hrs. The viosterol was actually administered as a 10,000×preparation but the dosage was calculated as cc./kg.m. of 100 X. Total, 1498 cc., or 93.6 cc./kg. of original weight. It will be noted that there was at first a steady, consistent rise in the rate over five days, with considerable variation thereafter, but with an average rate well above basal. The sudden sharp fall that occurred after the 30th day was at that time interpreted as an indication of established tolerance, consequently the dose was doubled on the 34th day, but a new preparation was used. Subsequently it was found that the first preparation had deteriorated in potency.

With the higher dosage the rate promptly increased again, finally, on the 42nd day, reaching the high point of plus 85 per cent. With discontinuance of administration after the 43rd day the rate gradually declined to a normal level on the 52nd day. With a still larger dose of 3 cc., begun on the 54th day, the rise in rate was much less pronounced. By this time the animal was obviously in a toxic state so that the subsequent results are of doubtful value. Death in coma occurred on the 89th day.

As mentioned above, the calculations of surface area on the basis of weight alone is of questionable validity. In this chart the calculation on the basis of weight at the time administration was begun (16.3 kg.) gave a surface area of 0.72 sq.m. and the higher graph is based on this figure. The

cross-hatched area indicates the rate calculated on daily weight. At the termination of the experiment the dog weighed 11 kilos, from which the formula gives a surface area of 0.554 sq.m. This means a loss of 32.5 per cent in weight and 23 per cent in area. It is scarcely conceivable that the figures for variation of surface area can be correct. Hence the above statement that the true rate probably lies somewhere between the two. Except where indicated the calculations are based on the actual weight of the animals on that day. This discrepancy does not, however, invalidate the results in the early stages before weight loss became pronounced. In fact, in the first period, the high rate, while accompanied by some irregularity was not accompanied by any consistent weight loss. Up to the 48th day this animal showed considerable increase in appetite so that the weight loss was not due to lessened food consumption.

In all animals, at autopsy, it was found that all visible stores of body fat were exhausted. This fact suggests, therefore, that the weight loss is due in the early stages to increased consumption of body fat. As will be seen in other cases, the time of beginning weight loss varied. When delayed there was invariably an increase in food consumption. It seems possible, therefore, that the delay depended on the extent to which food consumption counterbalanced fat loss.

The blood studies in this case do not confirm the common statement that the inorganic phosphorus is increased by ergosterol. While there are high values at certain points they are not consistently so. This is true of all of the animals in this series. The values for potassium are likewise widely variable so that it cannot be concluded at present that the blood concentration of this substance is affected in a constant manner.

The blood calcium did not show any pronounced change until the 31st day and had not returned to a normal level in more than three weeks after the last dose.

In case of Number 24 (Fig. 2) 9 large doses—4.8 cc./kgm.—were injected over a period of 11 days. Total 518.4 cc., or approximately 43.2 cc./kgm. of original weight. The metabolic rate was immediately increased and sustained through 5 days after the last injection, after which it fell abruptly to basal. Subsequent fluctuations cannot be evaluated. No pronounced weight loss occurred until the second day of the afterperiod. Appetite was good until the 32nd day, after which there was little food consumed.

In Number 25 (Fig. 3) the initial dose was only 3.7 cc./kgm. daily, but the response was immediate and sustained. After 8 days the dose was re-

duced to 0.7 cc./kgm. While there was some fluctuation in the rate, the average level continued high, approximately plus 33 per cent. With further reduction to 0.35 cc./kgm. the rate was still sustained at a definitely high level to the end of the experiment. Total viosterol 544.6 cc. or 38.4 cc./kgm. original weight.

There was no increase in inorganic phosphorus and no immediate increase in blood calcium. Weight loss was not as great as in either of the preceding animals, the final loss being slightly less than 13 per cent.

Number 26 (no chart) was given 2.3 cc./kgm. daily over a period of 12 days, and 0.6 cc./kgm. over 5 days. Total 260 cc. or 30.6 cc./kgm. The metabolic rate increased immediately and remained about +40 per cent. There was no weight loss until the tenth day of administration. The final weight was 7.2 kilos. as against 8.5 kilos. initial weight, approximately 15 per cent loss. The results in this case and in Number 24 are comparable in every respect except that the experiment was terminated at an earlier date in the case of Number 26.

Number 29 (no chart) received 7.25 cc./kgm. daily over a period of 13 days, but in spite of the high dose the average level of metabolic rate was only +20 per cent. The dose was now reduced to 1 cc./kgm. daily over 13 days with the result that the rate increased promptly to +30 per cent and two days later to +50 per cent. The average during this period was approximately +35 per cent. This response to a lower dosage has been noted in several cases and is one of the puzzling features of this work. At present a possible explanation seems to be that the animals had become sensitized. An alternative explanation is that the heavy administration resulted in storage of ergosterol in the body so that some of it was liberated and acted along with the smaller dose subsequently administered.

The fact that the early metabolic level was not as high on high dosage as in the other cases on lower dosage confirms Thöenes' claim that there is no quantitative relation between dose and metabolic rate. Still further confirmation is found in others of our experiments.

Blood calcium rose from 10.20 mgm. to 29.10 on the 21st day and was still 29.20 on the 29th day. Phosphorus fell from 6.01 mgm. to 3.24 although on the 21st day the figure was 8.52. Potassium was practically constant throughout.

It is obvious that the rise in metabolic rate cannot be correlated directly with any other factor studied except in the most general way.

That the response is not always prompt is illustrated by Number 30 (Fig. 4). On the 9th day of observation this dog received a daily dose of

2.5 cc./kgm. but it was not until the 15th day that the metabolic rate increased 30 per cent. On the 16th day the dose was reduced to 0.8 cc./kgm. but a rate of +38 per cent was maintained through three days. Because of a technical difficulty no further metabolic rate determinations were made on this dog. Beginning with the 20th day the dose was further reduced to 0.45 cc./kgm. daily. All observations were discontinued after the 30th day (September 15, 1930).

No significant weight loss occurred until the 18th day. The final weight was 9 kilos. as compared to 12.3 at the beginning. This weight was maintained for about a month when there began a slow recovery until on December 13, 1930, the weight was 15.4 kgm. During this period of recovery, the metabolic rate was very high, gradually falling from +50 per cent to basal by December 15 (91st day). Again because of a technical difficulty it was necessary to discontinue metabolic rate determinations.

On January 8, 1931, was begun a balance experiment on this dog, part of the results of which are shown in Figure 5. Urine volume was increased in the after period. Urine phosphorus decreased slightly but only as phosphorus consumption diminished, a good balance being maintained throughout.

Urine Ca elimination was at a low level when administration was begun on the 32nd day but on the 37th day a progressively increased output became apparent, which continued 5 days after the last dose.

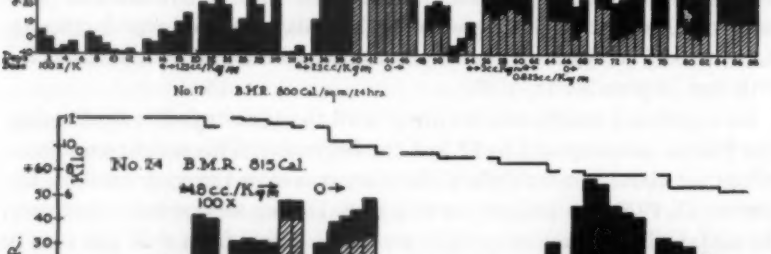
Blood Ca was increased progressively from 11.20 mgm. to 24.5 on the 4th day after the last dose, falling to 15.87 three days later but, strangely enough, rising again in three days to 23.30 mgm.

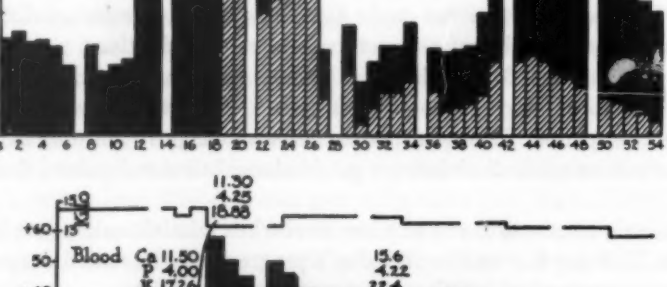
Weight loss was quite rapid after the 45th day, on which the highest urine Ca was found, the final figure being 12 kilos. nine days later. To what extent weight loss and high urine Ca output may be directly correlated remains to be determined.

In case of Number 35 (no chart) an effort was made to select a minimal effective dosage. On the 21st day 0.5 cc./kgm. daily was given but the metabolic rate showed no increase until the 25th day. There was no significant sustained weight loss. Urine P was slightly decreased during the period of administration. Urine Ca was not greatly affected, nor was the Ca balance altered. Total Ca elimination was decreased during the period of injection and slightly increased in the after period over the average of the foreperiod. It seems probable therefore that, for this dog, the dosage was about the threshold of toxicity.

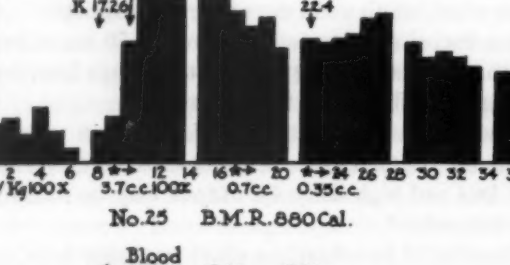
Another experiment of the same kind was done on Number 27 (Fig. 6).

No. 17 B.M.R. 800 Cal./sq.m./24 hrs.

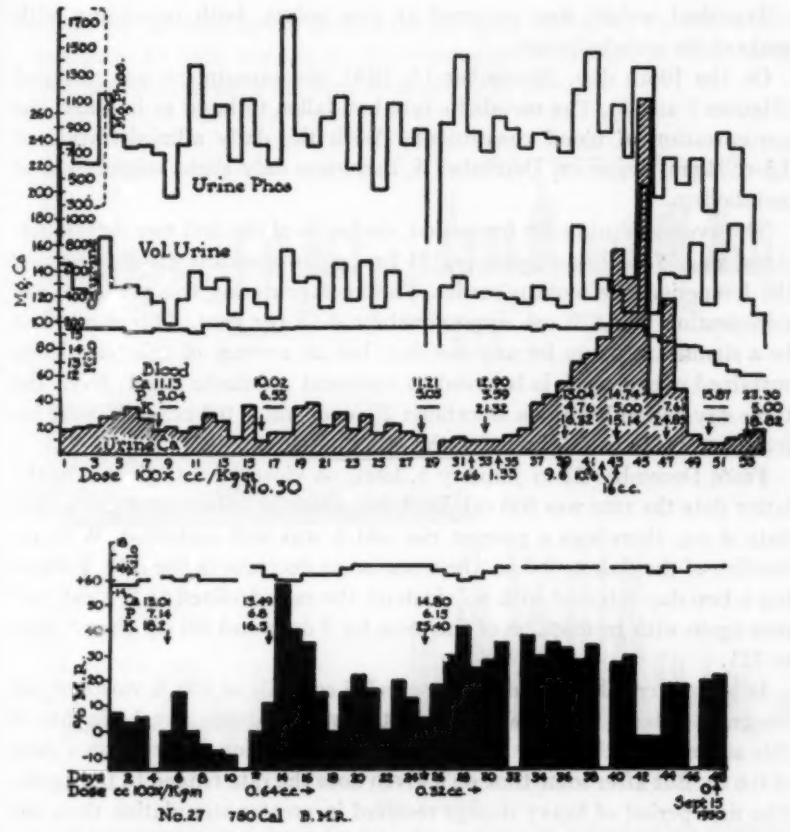



No. 25 B.M.R. 880 Cal.




No. 30 B.M.R. 830 Cal.




In the above Figures 1 to 6 inclusive, ★ indicates the date on which a given dosage was begun, e.g. Fig. 1, 16th day. The number of cc. per kilogram is shown by the figure accompanying. The arrow following indicates that that dose was repeated each day until another legend appears. A zero followed by an arrow indicates that on that date *et seq.*, no viosterol was administered.

Beginning with the 12th day 0.64 cc./kgm. daily promptly increased the metabolic rate 57 per cent. There were, however, three periods of subsidence to basal on the 18th, 22nd, and 25th days, which suggests that the dose was not sufficient to maintain a high metabolic level. This conclusion, however, is called into question when it is seen that half of this dose did sustain a high level, except for one remission through the 48th day.

Transient weight loss occurred at two points, both coinciding with peaks of the metabolic rate.

On the 106th day, November 13, 1930, the experiment was resumed (Figures 7 and 8). The metabolic rate had fallen to basal as had also the concentration of blood constituents. With the daily administration of 0.5 cc./kgm. begun on December 3, there was only slight stimulation of metabolism.

The average during the foreperiod, exclusive of the first two determinations, was 778 cal. per sq.m. per 24 hrs., approximating closely that for the foreperiod five months earlier. The average during this period of administration was 870 cal. approximately +15 per cent. This would not be a significant figure for any one day, but an average of this magnitude sustained over a week is believed to represent a definite effect. Even the three day period begun on December 20, over which 0.3 cc. daily were injected, seems to have stimulated the rate to some extent.

From December 22 to January 5, 1931, no viosterol was given. On the latter date the rate was 800 cal. With the administration of 0.6 cc. on this date *et seq.* there was a prompt rise which was well sustained. With reduction of the dose to 0.3 cc. there was some decrease in the rate. Following a two day interval with no viosterol the rate declined to 811 cal. but rose again with resumption of this dose for 3 days and fell off after 5 days to 751.

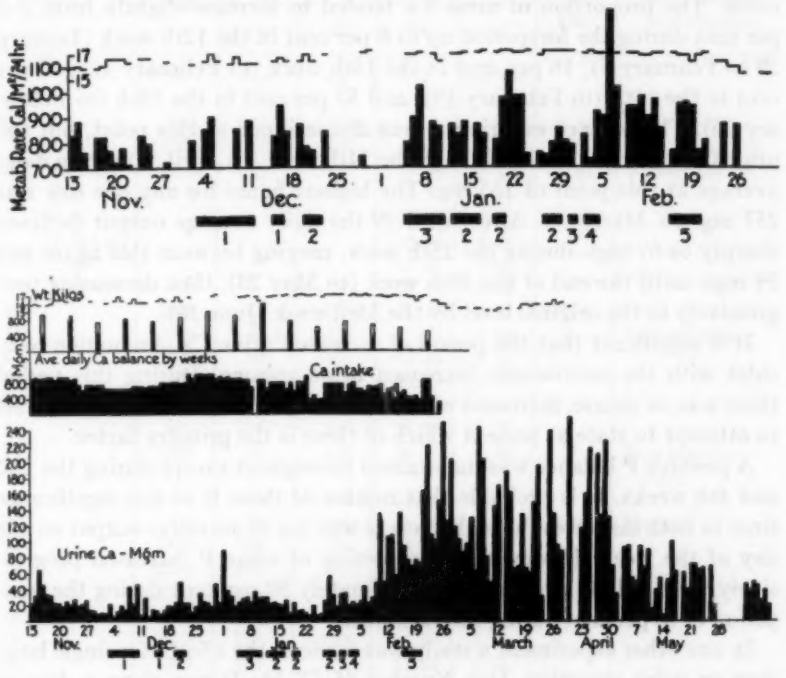
It is apparent that as small a dose as 0.3 cc. daily of 100 X viosterol per kilogram of body weight was sufficient to stimulate the metabolic rate of this animal, but that only slightly greater stimulation occurred on a dose of 0.6 cc. But after some time on a given dose the rate tended to fall again. The first period of heavy dosage resulted in greater stimulation than did the second.

The dog gained weight until the last period of administration, which terminated on February 20, and did not recover fully until two months later.

Blood Ca reached the high point of 18.59 mgm. on March 6, 13 days after the last dose, and progressively declined to 10.12 on July 7 except for one unexplained elevation to 13 mgm. on March 20.

The daily average urine volume decreased slightly during the 4th and 5th weeks (December 4 to December 18) but increased again during the 6th week (to December 25). Through the 7th and 8th weeks the foreperiod average was maintained. But during the 9th, 10th, 11th, 12th, 13th and 14th weeks, which correspond to the period of administration

begun on January 5, the daily average again decreased by about 50 cc. But with the 15th week (Feb. 20) began a great increase in urine output that persisted through the 32nd week, or up to June 25.




Figs. 7 and 8

The numbered black dashes in the lower line on the charts indicate periods of injection of viosterol. The same amount was injected daily over the number of days included, except as explained below. The numbers under the dashes refer to the following:

1. 0.5 cc. 100 X viosterol per kilogram daily.
2. 0.3 cc. 100 X viosterol per kilogram daily.
3. 0.6 cc. 100 X viosterol per kilogram daily.
4. 4.5 cc. 100 X viosterol per kilogram daily first day.
   6 cc. 100 X viosterol per kilogram daily second day.
5. 3.5 cc. 100 X viosterol per kilogram Feb. 16.
   5 cc. 100 X viosterol per kilogram Feb. 17.
   11.7 cc. 100 X viosterol per kilogram Feb. 18, 19, 20.

The upper interrupted graph gives the body weight. In Fig. 8 the daily Ca intake is shown as corresponding to the dates on the abscissa. The third tier shows the daily average Ca balance for the week corresponding to the dates. The black portion of the column shows the average total Ca output, the clear space the Ca retention, the entire column, Ca intake. In the 2nd, 4th and 9th weeks the intake is shown by the white line through the black columns.

A good positive Ca balance was maintained throughout except during the 4th and 9th weeks. In the latter case there was an extraordinarily high average daily output of 1.22 gms. of which 1.189 gms. was by the intestinal route. The proportion of urine Ca tended to increase slightly from 2–3 per cent during the foreperiod up to 6 per cent in the 12th week (January 29 to February 5); 16 per cent in the 13th week (to February 12); 30 per cent in the 14th (to February 19); and 50 per cent in the 15th (to February 26). The balance experiment was discontinued at this point, but the urine Ca continued to rise through the 24th week (to April 30) with a daily average at this point of 165 mg. The highest figure for any one day was 257 mg. on March 30. After April 29 the daily average output declined sharply to 69 mgs. during the 25th week, ranging between this figure and 59 mgs. until the end of the 28th week (to May 28), then decreasing progressively to the original level by the 33rd week (June 26).

It is significant that the period of increased urine Ca elimination coincides with the enormously increased urine volume. During this period there was, of course, increased water intake. But it would not be justifiable to attempt to state at present which of these is the primary factor.

A positive P balance was maintained throughout except during the 2nd and 4th weeks. It is probable that neither of these is of any significance since in both instances the high average was due to excessive output on one day of the week. However the proportion of urine P increased progressively from a daily average of approximately 50 per cent during the foreperiod to 78 per cent during the 15th week.

In one other experiment a study was made of the effect of a single large dose on urine excretion. Dog Number 45 (Table I) was given a dose of 4.75 cc. 10,000 X (8.6 kilos.), the equivalent of approximately 55.2 cc./kgm. of 100 X viosterol. A legitimate criticism of this experiment might be made in view of the short foreperiod. Nevertheless, the figures for this period are comparable to those obtained on other normal dogs. The injection was made at 9:00 a.m. on the third day (May 9, 1931) and the blood Ca and P followed at intervals for 7½ hours and daily thereafter. Another 1 cc. dose of viosterol was given on the 33rd day, June 4. The significant features are the enormous increase in urine Ca output and in urine volume. Urine P output was not significantly altered. This animal did not lose weight until after the 13th day, having gained slightly up to that time.

In general there was fairly close parallelism between urine volume and the content of Ca.

TABLE I
No. 45

| Date | Vol. | Urine | | Blood | | Wt. kilo. | Viosterol dosage and remarks |
|---|---|---|---|---|---|---|---|
| | | Ca | P | Ca | P | | |
| | cc. | mg. | mg. | mg. | per 100 cc. | | |
| 5–7 | 910 | 19 | 341 | | | 8.62 | |
| 8 | 1200 | 37.5 | 480 | | | | |
| 9 9:00 A.M. | 1450 | 20 | 421 | 11.44 | 5.12 | 8.62 | 4.74 cc. 10000X |
| 9:30 | | | | 10.84 | 4.44 | | |
| 10:30 | | | | 10.40 | 5.51 | | |
| 12:30 P.M. | | | | 10.40 | 6.45 | | |
| 2:30 | | | | 10.40 | 6.66 | | |
| 4:30 | | | | 10.40 | 4.77 | | |
| 10 | 1500 | 38 | 329 | 11.18 | 6.07 | | |
| 11 | 990 | 14.8 | 322 | 11.99 | 6.83 | | Mild diarrhea |
| 12 | 1300 | 60 | 512 | 15.80 | 7.27 | | |
| 13 | 1400 | 127 | 537 | 19.89 | 4.94 | | |
| 14 | 2250 | 275 | 595 | 14.00 | 6.06 | | |
| 15 | 970 | 217 | 500 | 15.75 | 4.79 | | |
| 16 | 270 | 43 | 131 | 16.85 | 7.48 | | Poor appetite, diarr. |
| 17 | 1200 | 77.5 | 318 | 16.75 | 4.15 | | |
| 18 | 580 | 65.5 | 196 | 15.70 | 5.33 | 8.70 | Livelier |
| 19 | 2700 | 309 | 513 | 15.30 | 4.33 | | |
| 20 | 2500 | 333 | 567 | 16.35 | 5.35 | 8.84 | |
| 21 | 1550 | 166 | 291 | 16.20 | 5.00 | | |
| 22 | 1600 | 183 | 200 | 12.84 | 5.58 | | |
| 23 | 1650 | 216 | 324 | 13.70 | 5.59 | | |
| 24 | 2500 | 263 | 506 | 15.29 | 6.61 | | |
| 25 | 500 | 64 | 169 | 14.88 | 5.06 | | |
| 26 | 3500 | 331 | 610 | 15.05 | | | |
| 27 | 2500 | 240 | 460 | 16.31 | 6.66 | | |
| 28 | 2500 | 374 | 606 | | | 8.00 | |
| 29 | 2500 | 367 | 416 | 21.60 | 5.63 | | |
| 6–2 | 2000 | 274 | 768 | 17.21 | 4.57 | | |
| 3 | 1000 | 160 | 478 | | | | |
| 4 | 2240 | 282.5 | 892 | 16.37 | 6.95 | | 1 cc. 10000 X |
| 5 | 1250 | 178.5 | 278 | 15.82 | 3.69 | | |
| 6 | 1530 | 197.5 | 241 | | | | |
| 7 | 1550 | 263 | 486 | | | | |
| 8 | 740 | 112 | 200 | | | | |
| 9 | 1300 | 112 | 263 | | | | |
| 10 | 1100 | | 160 | | | | |
| 11 | 1900 | | 500 | 18.30 | 3.72 | 7.48 | Poor appetite |
| 14 | 2500 | | 1030 | | | | |
| 15 | 1600 | | 540 | | 4.44 | 7.48 | |

In one human subject, a man of 65 kilos. weight, a balance experiment was done with a foreperiod of one week, after which 15 injections of 0.5 cc. 10,000 X viosterol were made over 16 days. On the 11th day only was the blood Ca significantly increased. A good N balance was maintained throughout with no significant alterations. There was definite P retention during two weeks and still greater retention during the remaining two days of injection, but during the 3 day after period, progressively increasing elimination.

Ca elimination was well balanced during the foreperiod, and during the first two weeks. There appeared a definite increase in the urinary fraction, both relative and absolute, with progressive retention. The patient experienced no subjective symptoms at any time and maintained constant weight.

## Discussion

In every experiment a significant increase in the resting, post-absorptive metabolic rate has been observed following viosterol administration. This invariable stimulation of the metabolic rate that occurs with any large dosage of irradiated ergosterol is difficult to explain at present. The response is immediate in most cases, but is sometimes delayed. Occasionally an animal has responded by a decreased metabolic rate after a decrease in the dose administered. The fact that the rate frequently decreases on continued administration might be interpreted as due to tolerance or to an altered state of metabolism accompanying toxicity.

The increased metabolic rate is apparently not to be correlated quantitatively with any single observed variable factor. Examination of the charts of rate and depth of respiration taken during each experiment does not indicate any regular increase in the respiratory rate but the depth was usually increased very definitely. Where the rate was increased it was not regularly correlated with a pronounced increase in metabolic rate. The greatest increase in oxygen intake was, with five exceptions, correlated with pronounced increase in depth of inspiration. The weight loss, which occurred even with increased food consumption, is also a difficult reaction to explain. In the later stages, of course, after toxic symptoms have developed, there is usually little or no food consumption.

Aside from the augmentation of the metabolic rate the Ca output is of special significance. When there is a relative increase in the urine fraction with no alteration of exchange it seems probable that this is due to increased absorption of Ca from the gut. But decreased ingestion with a

shift from feces to urine must be interpreted as the result of increased Ca mobilization. Where this phenomenon persists for weeks after administration has ceased, as in the case of Number 27, it must mean that the tissue deposits of Ca which we believe to occur are being eliminated by way of the kidney. Microscopic examination of the kidneys shows some injury to the tubular epithelium. It has been generally accepted that such injury alone will permit greater Ca loss so this factor may also be concerned. The results of analyses of the tissues of these animals for Ca and P will be reported in another paper.

While the effects on P excretion are less pronounced than on Ca, in general, it may be said that there is retention during mild administration, with a shift from feces to urine and perhaps some tendency to increased excretion in the after period.

In this connection it should be pointed out that the true after period cannot be determined since there is no method of determining when the ergosterol accumulated in the body is exhausted.

The great increase in urine volume accompanying increased urine Ca excretion, while not an invariable result, is nevertheless common enough to warrant attention. What is the primary import of this effect has not been determined. There was always increased water consumption, but whether this was a primary or secondary effect cannot be stated at present.

While Thöenes reports stimulation of nitrogen metabolism under ergosterol administration, neither our experiments nor those of Kern, Montgomery and Still (6), nor of Bauer, Marble and Claflin (1) confirm this finding. Our experiments are too inconclusive to warrant further discussion of this point at present. This question will be investigated further.

## Conclusions

1. Administration of large doses of irradiated ergosterol to normal dogs causes an increase in the metabolic rate that is not quantitatively related to any other observed factor.

2. During administration at a low level there is usually retention of Ca with a shift from feces to urine. On heavy or long-continued administration there is increased urine Ca elimination although a good balance may be maintained.

3. To a lesser extent the same holds for phosphorus excretion.

4. During the period of increased urine Ca elimination there is usually a great increase in urine volume.

5. There is no evidence of any effect on nitrogen metabolism.

6. It is believed that one of the early effects of heavy dosage with ergosterol is increased consumption of body fat.

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# THE CALCIFICATION OF TISSUES BY EXCESSIVE DOSES OF IRRADIATED ERGOSTEROL*

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THE deposition of calcium in various tissues after excessive dosage of irradiated ergosterol has been generally observed by those engaged in investigation of this problem, with the exception of Cartland, Speer, and Heyl (1) and Tavares (2). Duguid (3), Spies and Glover (4), Laas (5), Hoyle (6), Simmonet and Tauret (7), Huebner (8), Smith and Elvove, (9), Schoenholtz (10), Holtz and Von Brand (11), Herzenberg (12), Schiff (13), Comel (14), and Light, Miller, and Frey (19) have reported calcium deposition in various organs up to 100 times the normal content of such tissues.

In the course of investigations on the effects of viosterol on normal dogs, various tissues were analyzed for calcium and phosphorus content. The details of the experiments to which the animals were subjected are given in another paper (Reed, Thacker, Dillman and Welch, 15), but a summary is given in Table I, with the exception of Numbers 27 and 30, in which cases the procedure was varied as described below.

Number 27 received a total of 225.8 cc. of viosterol, 100 X, over 34 days, or 15.36 cc. per kilo. of body weight. This part of the experiment was terminated on September 15, 1930. On December 3 the injection of viosterol was resumed. During the next 80 days a total of 1004.4 cc. of viosterol was injected, or 62 cc. per kilogram of initial body weight. This dog was not killed until July 7, 1931, about 4 months after the last injection.

Number 30 received a total of 315.5 cc. over a period of 22 days, or 25.65 cc. per kilogram of original weight. This period terminated on September 15, 1930, and the dog was allowed to recover until February 8, 1931. Over the next 12 days a total of approximately 870 cc. of viosterol 100 X was

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injected, or 56.5 cc. per kilogram. The dog died on the 23rd day after administration was begun, or 11 days after the last injection.

The irradiated ergosterol was administered by intravenous injection, as viosterol 10000 X, but in all cases the dosage is calculated as cubic centimeters of 100 X per kilogram of original weight. The fifth column in the table indicates the number of days each animal survived after the first injection, regardless of whether it died from the toxic effects or was killed.

The tissues to be analyzed were weighed immediately, then dried at 105°C. for three days and again weighed into vitrosil crucibles, and ashed at 800°C. The ash was then dissolved in 10 cc. of 20 per cent trichloracetic acid, and diluted to 25 cc. in a volumetric flask. Portions of this solution were used for calcium determination by the method of Shohl and Pedley (16) and for phosphorus by that of Fiske and Subbarow (17). The results as shown in Tables III and IV were so variable that it was deemed best to resort to a statistical treatment of the data. Accordingly, 13 apparently normal dogs were killed and the tissues analyzed. In addition, one dog, Number 31, received 2 cc. of corn oil daily over 19 days in order to rule out possible influences of the oil itself. This amount is far in excess of any volume of corn oil actually injected as viosterol. The figures for the tissues of Numbers 27 and 31 are not included in the statistical comparisons, although both are shown in the table. Not all tissues were included for some of the animals. This fact renders the statistical comparison somewhat less significant. Aside from the value of the comparative results, it is possible that a record of the tissue content of calcium and phosphorus in normal animals may be of some value to others.

Of the 12 tissues analyzed from 13 normal dogs, the mean Ca values ranged from 14.3 mgm. per 100 gms. of dried tissue in the spleen to 101.3 mgm. in the thyroid. The highest values for each tissue ranged from 25 mgm. in muscle (Numbers 69, 80) to 263 mgm. in the thyroid (Number 66). The lowest value found in any one tissue was 7 mgm. in the spleen (Numbers 69, 71). The greatest standard deviation was found in the thyroid.

The mean values for P content ranged from 152.3 mgm. in the skin to 1317 in the brain. The highest values for each tissue ranged from 34 mgm. in the skin (Number 74) to 1526 mgm. in the brain (Number 70). The largest standard deviation was found in the kidney.

Of the tissues of Number 31 that were examined, the Ca content was usually within the normal range for each tissue but generally well above the mean. However, in the left ventricle, muscle, and skin, the values were

well above the range for the normal group. The P content shows much the same variation, being markedly higher in the aorta than the mean of the normal group, but in all other tissues the P content was in the upper extreme of the range, except in the left ventricle. These differences are of no significance statistically.

In the dogs that had received irradiated ergosterol, every tissue examined showed a higher mean Ca content than in the control series, except Number 27. As will be seen on comparing Tables I, III and IV, there was no direct correlation between the calcium content and total amount of viosterol injected, the amount injected per kilogram of body weight, the number of days, or the size of individual injections. The variations in the viosterol series probably would have been less extreme if every animal had been treated in exactly the same way. But since the major investigation had to do with the mechanism of physiological action of viosterol, it was inevitable that the treatment would be varied. Furthermore, the progress of the experiments frequently conditioned the procedures. While no two animals *were* treated in exactly the same way, it is clear that wide

TABLE I

| No. | Initial wt. kilos | Total cc. vosterol 100X | cc. 100 X viosterol/ kgm. | Days | No. doses | |
|---|---|---|---|---|---|---|
| 5 | 10.4 | 520 | 50 | 62 | 18 | Died |
| 17 | 16 | 1498 | 93.6 | 73 | 42 | Died |
| 21 | 15 | 1143 | 76 | 43 | 16 | Died |
| 24 | 12 | 518.4 | 57.6 | 47 | 9 | Killed |
| 25 | 14.2 | 544.6 | 38.4 | 33 | 32 | Killed |
| 26 | 8.5 | 260.1 | 30.6 | 17 | 17 | Died |
| 29 | 11.7 | 1254.8 | 107.25 | 30 | 26 | Died |
| 30 | 12.5 | *870 | 56.5 | | | Died |
| 32 | 11.4 | 305 | 26.75 | 23 | 17 | Killed |
| 33 | 11.4 | 340 | 28.95 | 33 | 18 | Killed |
| 35 | 12.2 | 184.6 | 15.13 | 29 | 29 | Killed |
| 45 | 8.6 | 575 | 67 | 35 | 2 | Killed |
| 108† | 10.5 | 1700 | 161.9 | 9 | 6 | Died |
| 109‡ | 12.7 | 1750 | 137.8 | 8 | 8 | Died |
| 27 | 14.5 | * | | | | |

* Detailed discussion of the treatment of these two dogs is given in the text.

† Viosterol administered after a 10 day rest period following injection of 85 cc. of parathormone over 31 days.

‡ Viosterol administered after a 10 day rest period following injection of 94 cc. of parathormone over 18 days.

variations in tissue deposits of calcium and phosphorus would result, even if all the animals responded in the same way to a given treatment. However, it is apparent that there are wide variations in the individual animals' capacity to respond to viosterol administration, as has been pointed

TABLE II
Calcium, mgm. per 100 gm.

| Tissue | Normal mean a | Ergosterolized mean b | $E_\Delta = \sqrt{a^2+b^2}$ | $\Delta/E_\Delta$ | Probability |
|---|---|---|---|---|---|
| Adrenal | 37.6 ± 3.24 | 203.1± 81.9 | 81.9 | 2.02 | 0.04 |
| Aorta | 64.23± 6.84 | 175.3± 35.7 | 35.9 | >3 | <0.01 |
| Brain | 55.4 ±14 | 125 ± 16.7 | 21.8 | >3 | <0.01 |
| R. Ventricle | 2.27± 4.43 | 149.6± 37.69 | 37.9 | 2.9 | <0.01 |
| L.V. | 18.3 ± 1.64 | 220.4± 63 | 64 | >3 | <0.01 |
| Kidney | 55.6 ±15.9 | 671.6±236.3 | 236.8 | 2.6 | <0.01 |
| Liver | 17.9 ± 1.22 | 122.8± 45.1 | 45.5 | 2.33 | 0.02 |
| Lung | 88.6 ±13.2 | 338.4±160.2 | 165 | 1.514 | 0.13 |
| Muscle | 17.8 ± 1.16 | 64.8± 13.3 | 13.4 | >3 | <0.01 |
| Skin | 23.6 ± 1.95 | 69.8± 16.1 | 16.2 | 2.85 | <0.01 |
| Spleen | 14.3 ± 2.19 | 100.8± 21.26 | 21.4 | >3 | <0.01 |
| Thyroid | 101.3 ±22.9 | 509.3±144.66 | 147 | 2.77 | <0.01 |
| | Phosphorus, mgm. per 100 gm. | | | | |
| Adrenal | 595.8 ±30 | 648 ±109.18 | 113.3 | <1 | 0.64 |
| Aorta | 272.2 ±18.4 | 315.4± 13.1 | 28.07 | <1 | 0.12 |
| Brain | 1317.3 ±31.5 | 1018.3±130 | 164 | 1.82 | 0.07 |
| R.V. | 708.1 ±37.7 | 736.9± 42.36 | 50.6 | <1 | 0.61 |
| L.V. | 767.3 ±50.3 | 694.5± 49.2 | 70.4 | 1.34 | 0.27 |
| Kidney | 815.8 ±74.3 | 994 ±126.5 | 146.7 | 1.21 | 0.23 |
| Liver | 790 ±24 | 681.5±104.1 | 106.8 | 1.02 | 0.30 |
| Lung | 865 ±53.3 | 977.6±163.6 | 172 | <1 | 0.51 |
| Muscle | 699.3 ±43.6 | 536.7± 47.7 | 60.4 | 2.19 | 0.03 |
| Skin | 152.3 ±37.1 | 280 ± 50.5 | 62.5 | 2.05 | 0.04 |
| Spleen | 925.1 ±40.8 | 901.3± 59.4 | 72.12 | <1 | 0.74 |
| Thyroid | 554 ±57 | 712.6±119 | 132 | 1.2 | 0.23 |

$E_\Delta = \sqrt{a^2+b^2}$, $a$ = standard deviation of normal series.
$b$ = standard deviation of experimental series.
$\Delta/E_\Delta$ $\Delta$ = difference between means of the two series

out by Thöenes (18). Another factor which might alter the statistical evaluation somewhat is the failure to include in the analysis all of the tissues for each dog in the experimental group.

There were individual animals in the experimental series with values well within the normal range. For example, Number 35 approximated the

mean values of the normal group in every tissue except in the skin, spleen and thyroid. Number 108 also was well within the normal range for the adrenal, aorta, kidney, and skin, but all other tissues showed high values.

Number 26 had the highest Ca content of all animals examined in the adrenal, brain and lung; but was third in order in the aorta, the right ventricle, left ventricle, skin, and spleen; and second in order in the kidney and liver, while in muscle the figure was only slightly higher than the highest figure for the normal group. Yet, from Table I, it is seen that this dog received next to the lowest total amount of viosterol and fourth in ascending order of amount per kilogram.

Number 30 showed the highest values for the right ventricle, left ventricle, kidney, liver, spleen, and thyroid and the second highest values for the lung and muscle, whereas for the aorta, brain, and skin the values were within the normal range.

In general, then, there is a tendency toward a comparable degree of Ca deposition in the majority of tissues of any one animal, but this tendency is by no means invariable, as any amount that will induce toxic symptoms induces wide variations.

In case of Number 35, it was believed that the viosterol dosage was near the threshold of toxicity for this dog, as judged by the response of the metabolic rate and urine Ca excretion, and the steady maintenance of body weight, as described in the paper previously referred to (15). The Ca content of all tissues in this dog closely approximated the mean values for the normal series except in the skin, spleen and thyroid.

The values obtained for Number 27 were comparable to the mean values for the corresponding tissues in the normal group, except in liver and muscle. In view of the fact that this dog lived approximately four months after the last viosterol administration, it seems probable that whatever excess Ca deposition occurred must have been removed during this period. Judging from the other animals, such deposition must have occurred at some time. It is not clear why the liver, particularly, and muscle to a lesser degree, retained such high contents.

In Table II is shown the statistical treatment for each tissue. The standard error (S.E.) was determined by the formula $S.E. = S.D./\sqrt{n}$, where S.D. is the standard deviation. The figures in the fourth column were obtained by dividing the difference between the means of the two series by the factors in the third column. The last column of represents the probability of normally distributed values that would have positive or negative deviations exceeding the standard deviation in the ratios shown

TABLE III

NORMAL DOGS—Mgm. Ca per 100 gm. Dried Tissue

| No. | Adrenal | No. | Aorta | No. | Brain | No. | R.V. | No. | L.V. | No. | Kidney | No. | Liver | No. | Lung | No. | Muscle | No. | Skin | No. | Spl'n | No. | Thyroid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 66 | 68 | 66 | 118 | 71 | 202 | 66 | 87 | 31 | 38.4 | 70 | 177 | 72 | 28 | 67 | 221 | 31 | 59 | 31 | 42 | 74 | 32 | 66 | 263 |
| 67 | 50 | 68 | 114 | 31 | 116 | 31 | 48 | 73 | 30 | 72 | 100 | 81 | 27 | 68 | 135 | 69 | 25 | 74 | 36 | 67 | 21 | 68 | 239 |
| 81 | 47 | 31 | 103 | 70 | 100 | 74 | 46 | 66 | 28 | 31 | 72 | 80 | 26 | 71 | 120 | 80 | 25 | 78 | 35 | 65 | 19 | 67 | 231 |
| 69 | 40 | 72 | 81 | 67 | 83 | 80 | 36 | 80 | 24 | 66 | 70 | 31 | 24.9 | 72 | 103 | 67 | 20 | 72 | 31 | 78 | 19 | 74 | 119 |
| 80 | 40 | 65 | 80 | M | 55 | 73 | 35 | 78 | 23 | 73 | 67 | 67 | 19 | 80 | 97 | 74 | 20 | 81 | 30 | 80 | 16 | 45 | 110 |
| M | 37.6 | M | 64 | 80 | 49 | M | 27 | 72 | 21 | 80 | 62 | 73 | 18 | 73 | 90 | 66 | 18 | 67 | 24 | 68 | 15 | M | 101 |
| 73 | 37 | 80 | 60 | 74 | 43 | 72 | 33 | M | 18 | M | 55.6 | M | 17.9 | M | 88 | 72 | 18 | M | 23 | M | 14.3 | 71 | 80 |
| 71 | 36 | 67 | 57 | 68 | 42 | 81 | 23 | 67 | 16 | 74 | 55 | 65 | 17 | 31 | 87 | 81 | 18 | 69 | 22 | 77 | 14 | 78 | 78 |
| 65 | 32 | 70 | 56 | 69 | 42 | 69 | 22 | 70 | 15 | 78 | 55 | 70 | 17 | 66 | 75 | M | 17.8 | 80 | 22 | 81 | 14 | 80 | 71 |
| 68 | 32 | 73 | 54 | 65 | 41 | 78 | 18 | 74 | 15 | 81 | 50 | 68 | 15 | 70 | 64 | 70 | 17 | 65 | 21 | 73 | 13 | 64 | 57 |
| 78 | 32 | 81 | 51 | 73 | 40 | 68 | 16 | 65 | 14 | 71 | 46 | 71 | 15 | 74 | 62 | 78 | 16 | 70 | 21 | 66 | 10 | 73 | 52 |
| 70 | 29 | 69 | 44 | 66 | 36 | 70 | 15 | 81 | 14 | 65 | 43 | 69 | 15 | 69 | 51 | 68 | 15 | 73 | 19 | 70 | 8 | 72 | 49 |
| 74 | 24 | 74 | 43 | 78 | 35 | 65 | 14 | 68 | 13 | 67 | 35 | 74 | 15 | 65 | 48 | 73 | 15 | 71 | 18 | 69 | 7 | 81 | 43 |
| 72 | 22 | 78 | 40 | 81 | 35 | 71 | 13 | 67 | 13 | 68 | 31 | 78 | 13 | 78 | 46 | 65 | 13 | 66 | 15 | 71 | 7 | 70 | 40 |
| 31 | — | 71 | 37 | 72 | 24 | 67 | 13 | 71 | 12 | 69 | 29 | 66 | 10 | 81 | 42 | 71 | 12 | 68 | 13 | 31 | — | 31 | — |
| Mean | 37.6 | | 64.23 | | 55.4 | | 27.7 | | 18.3 | | 55.6 | | 17.9 | | 88.6 | | 17.8 | | | | 14.3 | | 101.3 |
| S.D. | 11.71 | | 24.65 | | 50.5 | | 15.96 | | 5.9 | | 57.4 | | 4.4 | | 47.9 | | 4.2 | | | | 7.93 | | 82.6 |
| S.E. | 3.24 | | 6.84 | | 14 | | 4.43 | | 1.64 | | 15.9 | | 12.2 | | 13.2 | | 1.16 | | | | 2.19 | | 22.9 |

Mgm. P. per 100 gm. Dried Tissue

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 81 | 800 | 31 | 730 | 70 | 1526 | 78 | 1008 | 78 | 1094 | 74 | 1112 | 73 | 990 | 78 | 1143 | 73 | 939 | 74 | 344 | 78 | 1198 | 78 | 802 |
| 68 | 736 | 80 | 398 | 72 | 1445 | 69 | 994 | 70 | 979 | 78 | 1073 | 80 | 897 | 69 | 1108 | 78 | 920 | 72 | 296 | 73 | 1185 | 73 | 801 |
| 74 | 725 | 78 | 342 | 66 | 1410 | 80 | 920 | 73 | 967 | 70 | 1020 | 81 | 870 | 80 | 1042 | 74 | 839 | 73 | 234 | 65 | 1180 | 81 | 796 |
| 65 | 630 | 81 | 338 | 31 | 1407 | 81 | 829 | 71 | 946 | 73 | 1015 | 69 | 854 | 68 | 1013 | 71 | 810 | 81 | 206 | 84 | 994 | 31 | 794 |
| 78 | 627 | 71 | 329 | 68 | 1399 | 31 | 760 | 81 | 845 | 72 | 1007 | 65 | 800 | 67 | 946 | 81 | 769 | 31 | 201 | 80 | 953 | 74 | 791 |
| 66 | 618 | 73 | 314 | 80 | 1398 | 71 | 721 | 74 | 781 | 69 | 970 | 68 | 790 | M | 865 | 72 | 739 | 78 | 188 | M | 925 | 80 | 745 |
| M | 595 | 72 | 290 | 74 | 1364 | M | 708 | M | 767 | 71 | 962 | M | 790 | 72 | 842 | 69 | 724 | M | 152 | 70 | 913 | 68 | 586 |
| 70 | 584 | M | 272 | M | 1317 | 66 | 707 | 69 | 760 | 81 | 874 | 67 | 782 | 70 | 841 | M | 699 | 65 | 116 | 69 | 871 | M | 554 |
| 80 | 575 | 65 | 268 | 67 | 1304 | 68 | 668 | 62 | 725 | M | 815 | 66 | 767 | 66 | 801 | 80 | 686 | 80 | 110 | 68 | 869 | 71 | 530 |
| 72 | 549 | 70 | 248 | 65 | 1299 | 65 | 656 | 72 | 682 | 80 | 795 | 78 | 742 | 65 | 778 | 67 | 625 | 68 | 92 | 71 | 850 | 65 | 448 |
| 69 | 541 | 68 | 242 | 78 | 1280 | 73 | 734 | 31 | 676 | 31 | 764 | 70 | 733 | 74 | 765 | 66 | 591 | 66 | 86 | 72 | 820 | 66 | 448 |
| 73 | 489 | 66 | 218 | 69 | 1230 | 74 | 632 | 66 | 580 | 66 | 554 | 71 | 715 | 71 | 710 | 31 | 520 | 67 | 84 | 66 | 814 | 72 | 374 |
| 71 | 481 | 71 | 197 | 71 | 1215 | 72 | 631 | 69 | 555 | 67 | 445 | 72 | 669 | 73 | 702 | 65 | 520 | 70 | 80 | 67 | 798 | 67 | 338 |
| 67 | 390 | 69 | 182 | 81 | 1130 | 70 | 596 | 80 | 548 | 68 | 425 | 74 | 662 | 31 | 645 | 68 | 520 | 71 | 77 | 81 | 682 | 70 | 300 |
| 31 | — | 67 | 173 | 73 | 1125 | 67 | 581 | 65 | 513 | 65 | 354 | 31 | 512 | 81 | 555 | 70 | 409 | 69 | 73 | 31 | — | 69 | 243 |
| Mean | 595.8 | | 272.2 | | 1317.3 | | 708.1 | | 767.3 | | 815.8 | | 790.0 | | 865 | | 699.3 | | 152.3 | | 925.1 | | 554.0 |
| S.D. | 108.4 | | 66.5 | | 113.5 | | 136.0 | | 181.6 | | 268.0 | | 86.8 | | 192.1 | | 157.5 | | 133.8 | | 147.3 | | 205.6 |
| S.E. | 30.0 | | 18.4 | | 31.5 | | 37.7 | | 50.3 | | 74.3 | | 24.0 | | 53.3 | | 43.6 | | 37.1 | | 40.8 | | 57 |

S.D. Standard Deviation
S.E. Standard Error

TABLE IV

VIOSTEROLIZED DOGS Mgm. Ca per 100 gm. dried tissue

| No. | Adrenal | No. | Aorta | No. | Brain | No. | R. V. | No. | L. V. | No. | Kidney | No. | Liver | No. | Lung | No. | Muscle | No. | Skin | No. | Spleen | No. | Thyroid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 26 | 777 | 32 | 484 | 24 | 260 | 30 | 517 | 30 | 969 | 30 | 3464 | 27 | 678 | 26 | 1860 | 24 | 227 | 21 | 241 | 30 | 238 | 30 | 1421 |
| 33 | 279 | 24 | 305 | 108 | 227 | 109 | 232.5 | 109 | 585 | 26 | 1214 | 30 | 591 | 30 | 1288 | 27 | 101 | 24 | 116 | 24 | 213 | 109 | 793 |
| 17 | 229 | 26 | 276 | 33 | 203 | 25 | 195 | 26 | 370 | 24 | 1148 | 26 | 265 | 25 | 846 | 30 | 100 | 26 | 96 | 26 | 186.6 | M | 509 |
| M | 203 | 109 | 188 | 17 | 148 | 26 | 184 | 25 | 364 | 109 | 1142 | 109 | 157 | 24 | 482 | 108 | 82 | 17 | 84.4 | 21 | 172 | 21 | 504 |
| 25 | 137 | M | 175 | M | 125 | 24 | 161 | 108 | 254 | 5 | 693 | 21 | 130 | 17 | 374 | 29 | 68 | 25 | 78.6 | 29 | 101 | 108 | 488 |
| 109 | 68.1 | 29 | 170 | 24 | 121 | 29 | 159 | M | 220 | M | 671 | M | 122 | 109 | 365 | 109 | 67 | M | 69 | 100 | 100 | 45 | 381 |
| 32 | 55 | 21 | 137 | 45 | 114 | M | 149 | 24 | 135 | 108 | 597 | 29 | 84 | M | 338 | 32 | 65.5 | 109 | 43.5 | 5 | 90 | 29 | 369 |
| 27 | 50.3 | 33 | 133 | 21 | 89.9 | 108 | 120 | 21 | 108.8 | 45 | 257 | 32 | 42.8 | 108 | 322 | 21 | 65.4 | 33 | 40.3 | 109 | 81.6 | 35 | 132 |
| 108 | 44.8 | 25 | 115 | 25 | 89 | 5 | 83.5 | 17 | 76.4 | 21 | 212 | 5 | 34.5 | 5 | 233 | M | 64 | 35 | 34.5 | 32 | 80 | 25 | 87 |
| 35 | 34.2 | 45 | 110 | 32 | 87.3 | 17 | 83.5 | 32 | 71 | 29 | 212 | 33 | 26.7 | 21 | 222 | 5 | 57 | 30 | 28 | 108 | 61 | 27 | 63.4 |
| 5 | — | 35 | 72.7 | 109 | 87 | 21 | 73.7 | 29 | 46 | 32 | 131 | 35 | 25.5 | 45 | 215 | 33 | 42 | 29 | 26.4 | 25 | 49 | 5 | — |
| 21 | — | 30 | 68.2 | 30 | 86 | 33 | 25.2 | 35 | 35.9 | 33 | 119 | 108 | 20.5 | 32 | 97.4 | 17 | 34 | 108 | 25.5 | 33 | 48.3 | 17 | — |
| 24 | — | 108 | 50 | 5 | 66.9 | 45 | 22.1 | 45 | 32 | 17 | 115 | 25 | 20.4 | 29 | 94.7 | 27 | 34 | 45 | 23.5 | 35 | 35.8 | 24 | — |
| 29 | — | 27 | 49.2 | 35 | 54.3 | 27 | 16.8 | 33 | 19.5 | 25 | 110 | 45 | 20 | 27 | 78.6 | 25 | 27 | 27 | 20.4 | 45 | 35 | 26 | — |
| 30 | — | 5 | — | 27 | 34.2 | 32 | — | 27 | 17.3 | 35 | 52 | 17 | — | 33 | 68 | 45 | 24.8 | 5 | — | 27 | 23.6 | 32 | — |
| 45 | — | 17 | — | 29 | — | 35 | — | 5 | — | 27 | 47.2 | 24 | — | 35 | 61 | 35 | 14 | 32 | — | 17 | 33 | 33 | — |
| Mean | 203.1 | | 175.3 | | 125 | | 149.6 | | 220.4 | | 671.6 | | 122.8 | | 338.4 | | 64.8 | | 69.8 | | 100.8 | | 509.3 |
| S.D. | 231.6 | | 123.6 | | 60.3 | | 130.4 | | 227.2 | | 883.8 | | 156.1 | | 599.4 | | 50.0 | | 59.9 | | 76.08 | | 409.4 |
| S.E. | 81.9 | | 35.7 | | 16.7 | | 37.7 | | 637 | | 236.3 | | 45.1 | | 160.2 | | 13.0 | | 16.1 | | 21.26 | | 144.66 |

Mgm. P. per 100 gm. Dried Tissue

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 1137 | 32 | 473 | 33 | 1968 | 30 | 1035 | 109 | 1009 | 109 | 1705 | 5 | 1410 | 45 | 2440 | 26 | 784 | 30 | 761 | 5 | 1335 | 109 | 1190 |
| 19 | 936 | 26 | 446 | 21 | 1511 | 24 | 866 | 21 | 841 | 45 | 1650 | 21 | 1333 | 26 | 2156 | 5 | 756 | 21 | 446 | 26 | 1272 | 45 | 1079 |
| 26 | 767 | 24 | 349 | 26 | 1488 | 33 | 819 | 26 | 834 | 26 | 1503 | 45 | 893 | 24 | 1439 | 24 | 670 | 25 | 368 | 24 | 996 | 29 | 937 |
| 27 | 764 | 29 | 339 | 24 | 1241 | 21 | 775 | 45 | 822 | 5 | 1394 | 26 | 690 | M | 977 | 108 | 670 | 26 | 343 | 32 | 994 | 08 | 765 |
| 33 | 762 | 33 | 338 | 17 | 1220 | 27 | 754 | 33 | 797 | 24 | 1282 | M | 681 | 21 | 950 | 27 | 645 | 29 | 324 | 45 | 993 | 21 | 723 |
| 32 | 674 | M | 315 | 27 | 1192 | 25 | 747 | 32 | 788 | M | 994 | 27 | 644 | 17 | 882 | 21 | 638 | M | 280 | 27 | 986 | M | 712 |
| M | 648 | 35 | 273 | 108 | 1169 | M | 736 | 108 | 742 | 21 | 946 | 29 | 630 | 32 | 796 | 32 | 589 | 17 | 221 | 21 | 940 | 25 | 497 |
| 109 | 500 | 25 | 227 | 109 | 1164 | 26 | 729 | M | 694 | 108 | 941 | 109 | 601 | 5 | 793 | 33 | 570 | 24 | 208 | M | 901 | 35 | 388 |
| 108 | 216 | 30 | 215 | M | 1018 | 108 | 659 | 29 | 641 | 32 | 905 | 32 | 523 | 25 | 690 | 45 | 564 | 35 | 181 | 109 | 870 | 27 | 383 |
| 35 | 196 | 45 | 198 | 5 | 924 | 109 | 644 | 24 | 602 | 29 | 826 | 33 | 504 | 33 | 690 | 25 | 537 | 109 | 169 | 33 | 832 | 30 | 125 |
| 5 | — | 27 | 197 | 25 | 731 | 5 | 560 | 17 | 583 | 30 | 507 | 30 | 452 | 27 | 682 | M | 536 | 33 | 137 | 25 | 801 | 5 | — |
| 21 | — | 109 | 193 | 32 | 554 | 17 | 560 | 25 | 523 | 27 | 464 | 25 | 396 | 30 | 644 | 109 | 505 | 45 | 101 | 29 | 756 | 17 | — |
| 24 | — | 108 | 182 | 35 | 492 | 45 | 552 | 30 | 461 | 35 | 441 | 35 | 392 | 108 | 578 | 29 | 440 | 108 | 101 | 108 | 703 | 24 | — |
| 29 | — | 5 | — | 45 | 466 | 29 | 495 | 35 | 446 | 25 | 415 | 108 | 351 | 109 | 565 | 30 | 365 | 27 | 42 | 30 | 629 | 26 | — |
| 30 | — | 17 | — | 30 | 312 | 32 | — | 27 | 201 | 17 | 409 | 17 | — | 29 | 550 | 35 | 275 | 5 | — | 35 | 597 | 32 | — |
| 45 | — | 21 | — | 29 | — | 33 | — | 5 | — | 33 | — | 24 | — | 35 | 509 | 17 | 151 | 32 | — | 17 | — | 33 | — |
| Mean | 648 | | 315.4 | | 1018.3 | | 736.9 | | 694.5 | | 994.0 | | 681.5 | | 977.6 | | 536.7 | | 280.0 | | 901.3 | | 712.6 |
| S.D. | 309 | | 43.5 | | 471.3 | | .46.6 | | 172.6 | | 456.1 | | 360.6 | | 612.1 | | 178.4 | | 174.8 | | 214.4 | | 339.3 |
| S.E. | 109.2 | | 13.1 | | 130.0 | | 42.4 | | 49.2 | | 126.5 | | 104.1 | | 163.6 | | 47.7 | | 50.5 | | 59.4 | | 119 |

S.D. Standard Deviation
S.E. Standard Error

in the fourth column of Table II as determined from Fisher's table (20). According to this author, unless the factor $\Delta/E_{\Delta}$ is 3 or more, the observed differences between the mean values are not significant statistically. By this criterion, only the aorta, brain, right ventricle, left ventricle, muscle, and spleen showed mean differences for Ca that are significant, although the probability is 1 per cent or less in the kidney, skin, and thyroid. Ranging from 2 to 4 per cent are the adrenal and liver, the lung alone showing a high percentage of probable variation beyond the standard deviation. However, it appears that only in case of the lung is there a probability of any great magnitude that the difference between the two series is the result of chance; here the probability is 0.13 or 13 chances in a hundred. In view of the fact that the experimental series does not represent homogeneous sampling even this figure does not entirely invalidate a conclusion that the experimental treatment was responsible for the definitely higher mean value.

It will be noted in Tables III and IV that in certain tissues the Ca content for a few animals is so far at variance with the range of the rest of the series as to be considered of a different magnitude. For example, the adrenal of No. 26 showed a value of 777, nearly three times as large as the next highest figure. These occasional variants necessarily increase the standard deviation and error. Excluding this one figure, the mean becomes 121, standard deviations, $90.6 \pm 34.16$, $\Delta/E_{\Delta}'$, 2.4 and the probability is reduced from 4 per cent to 1 per cent.

However, there seems to be a fair probability that a larger number of animals in each series would show more significant differences, so that it seems to be a valid conclusion.

The mean reduction in the P content of the brain, according to Table II, results in a probability of 0.07 or 7 chances in 100 that the difference is due to chance. The probability for muscle and skin are 0.03 and 0.04 respectively or 3 and 4 chances in 100 that the differences are due to random sampling. In all other tissues the probabilities are so high that in the absence of a larger series it must be questioned whether irradiated ergosterol has influenced the P content.

In neither series was there any close correlation between the Ca and P content of a given tissue.

The significance of Ca deposition in the tissues cannot be evaluated at present. Microscopic examination showed degenerated cells in some tissues but this was not a constant finding except in the kidney. Here one gains the impression that cellular injury occurred first, in which case Ca dep-

osition probably occurred as a direct result of such injury. But this does not account for the findings in other tissues because of the infrequency of microscopic evidence of injury, even where extensive Ca deposition had occurred.

## Summary and Conclusions

1. Analyses were made for Ca and P content of 12 tissues from 13 normal dogs and from 14 dogs that had received, by intravenous injection, toxic doses of irradiated ergosterol in the form of viosterol, 10000 X.

2. Statistical treatment of the data shows that the Ca content of any tissue may be significantly increased by viosterol administration, although wide variations may occur, certain tissues showing no increase.

3. The magnitude of the increase in Ca content is not correlated with the viosterol dosage but seems to depend on some undetermined individual factor or factors.

4. The P content, while widely variable among individual animals, was affected by viosterol administration in a much less constant manner, if at all.

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# VARIATION OF BASAL METABOLIC RATE PER UNIT SURFACE AREA WITH AGE

## II. THE PUBERTAL ACCELERATION

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Received for Publication—August 11, 1932

THE variation of the basal metabolic rate per unit surface area with age during the pubertal period requires special definition.

Originally Du Bois (1) found the calories per square meter per hour of several prepubescent boys above the trend located by reported normals for preceding and subsequent years. On this basis he hypothesized a prepubertal rise in basal metabolism (2, 3). Subsequent to the establishment of puberty the same boys gave rates in agreement with the trend (4).

But Benedict and Talbot (5) observed no tendency in either sex toward a prepubescent divergence of the basal metabolic rate from its earlier rectilinear trend.

Yet the determinations of Benedict and Hendry (6) of the minimum metabolic rates of groups of sleeping girls of closely similar ages, supplemented by those of Benedict (7), plot its decline as following a fairly symmetrical sigmoid curve with its inflection in the fifteenth year, which is the earliest year for which all the members of a group have menstruated. The individual determinations of Bedale (8) on girls awake but still abed agree with these group determinations (9).

Similarly the trend of the means of the yearly averages of MacLeod (10) and of Blunt et al. (11) of the basal metabolic rate of girls under comparable clinical conditions, weighted with the number of subjects on which each is based, pursues a skew sigmoid curve with its inflection in the fifteenth year.

Further, Göttche (12) found the earlier gradual decrease in the basal metabolic rate for both sexes (recalculated for surface area (24): Table I, Chart 1) interrupted by a sharp prepubertal and pubescent increase, followed by a rapid post-pubertal recession. And Lax and Petényi (13) found the basal metabolic rate during the pubertal period to conform to the Du Bois curve with its prepubertal rise (1–4).

In a very large series of extremely accurate determinations, obtained by averaging the results of several duplicate tests which showed no downward

trend for each subject, Bierring (14) found an excess of relatively high basal metabolic rates during the fourteenth and fifteenth years in boys with manifest signs of puberty. The median trend of these data (Chart 2) shows a retardation of the decrease in the basal metabolic rate during the

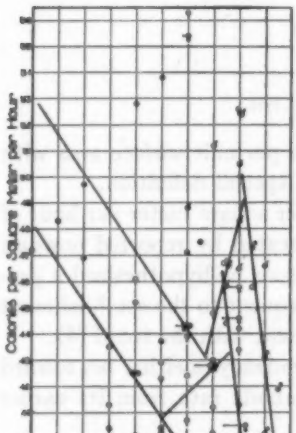


CHART 1

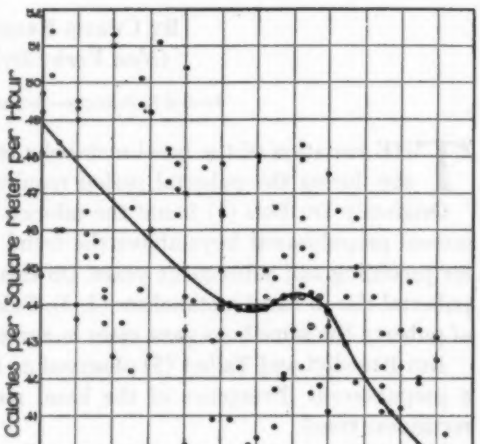


CHART 2

CHART 1. Basal metabolic rate of boys and girls (after data of Göttche). ⊙ represents boys, ○ represents girls, prior to puberty. ♂ and ♀ represent respectively boys and girls subsequent to puberty. ♂ without its spear-head, and ♀ without its cross-bar, represent respectively boys and girls during pubescence. ♂ with its shaft projecting from the center of the circle and without the spear-head represents boys immediately before pubescence. The lines and arrow-heads to the left of ♀ point to the time of the first menstruation. The graphs represent the successive trends before, during, and after puberty. During the periods in which the graphs overlap separate standards would seem from these data to be indicated according to the pubertal status of the subject.

CHART 2. Basal metabolic rate of boys (after Bierring). Scatter diagram. Graph of median trend. ○ represents nine-point moving median.

thirteenth year, a distinct rise in the fourteenth year, and an accelerated decline subsequently.

By making successive determinations on a number of children of each sex at intervals during the pubertal period, Topper and Mulier (15) (re-

TABLE I
CALORIES PER SQUARE METER OF BODY SURFACE PER HOUR DURING PUBERTY (AFTER GÖTTCHE)

TABLE I

CALORIES PER SQUARE METER OF BODY SURFACE PER HOUR DURING PUBERTY (AFTER GÖTTCHE)

| Sex | Age | Apubertal | | | | Pubescent | | | | Pubertal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Height | Weight | Area | Rate | Height | Weight | Area | Rate | Height | Weight | Area | Rate |
| | yrs. | cm. | kg. | sq. m. | cal. | cm. | kg. | sq. m. | cal. | cm. | kg. | sq. m. | cal. |
| Male | 6 | 86 | 11.5 | 0.52 | 61.4 | | | | | | | | |
| | 7 | 114 | 22.2 | 0.82 | 48.3 | | | | | | | | |
| | 8 | 131 | 28.1 | 1.02 | 46.9 | | | | | | | | |
| | | 119 | 22.2 | 0.86 | 49.7 | | | | | | | | |
| | 9 | 128 | 25.1 | 0.95 | 40.4 | | | | | | | | |
| | 10 | 130 | 24.7 | 0.96 | 42.5 | | | | | | | | |
| | | 126 | 21.6 | 0.88 | 52.8 | | | | | | | | |
| | 11 | 137 | 30.4 | 1.08 | 43.7 | | | | | | | | |
| | | 123 | 20.2 | 0.86 | 53.8 | | | | | | | | |
| | 12 | 124 | 25.3 | 0.94 | 44.3 | 147 | 39.3 | 1.27 | 48.8† | | | | |
| | | 133 | 27.1 | 1.00 | 47.1 | | | | | | | | |
| | 12½ | 135 | 26.1 | 1.00 | 47.5 | | | | | | | | |
| | 13 | | | | | 162 | 33.0 | 1.27 | 51.2 | | | | |
| | 13½ | | | | | 142 | 30.2 | 1.11 | 44.4 | | | | |
| | | | | | | 165 | 45.7 | 1.48 | 45.8 | | | | |
| | | | | | | 150 | 35.0 | 1.23 | 46.6 | | | | |
| | | | | | | 138 | 29.7 | 1.08 | 46.7 | | | | |
| | 14 | | | | | 157 | 42.2 | 1.37 | 36.2 | | | | |
| | | | | | | 152 | 38.4 | 1.29 | 46.5 | | | | |
| | | | | | | 142 | 31.9 | 1.13 | 48.3 | | | | |
| | | | | | | 149 | 36.0 | 1.24 | 50.5† | | | | |
| | | | | | | 142 | 33.0 | 1.16 | 52.4 | | | | |
| | 14½ | | | | | | | | | 139 | 30.5 | 1.10 | 45.8 |
| | | | | | | | | | | 141 | 39.5 | 1.24 | 47.3 |
| | 15 | | | | | 133 | 33.5 | 1.11 | 46.6 | 150 | 38.3 | 1.28 | 40.2 |
| | | | | | | | | | | 159 | 51.5 | 1.51 | 43.1 |
| | 15½ | | | | | | | | | 160 | 48.8 | 1.49 | 41.9 |

† Prepubertal.

TABLE I (*continued*)

CALORIES PER SQUARE METER OF BODY SURFACE PER HOUR DURING PUBERTY (AFTER GÖTTCHE)

| Sex | Age | Apubertal | | | | Pubescent | | | | Pubertal | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Height | Weight | Area | Rate | Height | Weight | Area | Rate | Height | Weight | Area | Rate |
| | yrs. | cm. | kg. | sq. m. | cal. | cm. | kg. | sq m. | cal. | cm. | kg. | sq. m. | cal. |
| Female | 3½ | 100 | 14.6 | 0.63 | 47.9 | | | | | | | | |
| | 6 | 104 | 18.3 | 0.71 | 47.2 | | | | | | | | |
| | 9 | 130 | 28.0 | 1.01 | 41.3 | | | | | | | | |
| | | 131 | 24.1 | 0.96 | 43.5 | | | | | | | | |
| | 10 | 135 | 28.2 | 1.04 | 40.1 | 141 | 42.0 | 1.26 | 46.1 | | | | |
| | | 130 | 25.2 | 0.96 | 42.5 | | | | | | | | |
| | | 123 | 30.3 | 1.00 | 45.2 | | | | | | | | |
| | 11 | 134 | 29.2 | 1.05 | 40.2 | 150 | 33.5 | 1.21 | 39.2 | | | | |
| | | 145 | 30.0 | 1.13 | 40.4 | 139 | 42.5 | 1.26 | 42.3 | | | | |
| | | 141 | 33.0 | 1.16 | 42.9 | | | | | | | | |
| | 12 | 139 | 31.0 | 1.10 | 37.9 | 155 | 47.5 | 1.44 | 39.4 | 152 | 42.6 | 1.35 | 44.3 |
| | | 140 | 33.5 | 1.15 | 38.7 | 136 | 28.8 | 1.06 | 40.7 | 139 | 29.3 | 1.07 | 55.4 |
| | | 138 | 31.8 | 1.10 | 42.4 | 149 | 39.5 | 1.29 | 43.9 | | | | |
| | | | | | | 132 | 29.1 | 1.03 | 56.3 | | | | |
| | 13 | 137 | 30.6 | 1.09 | 43.0 | 146 | 31.8 | 1.16 | 38.7 | 156 | 42.7 | 1.39 | 42.8 |
| | | | | | | 139 | 31.0 | 1.10 | 40.2 | | | | |
| | | | | | | 161 | 38.5 | 1.36 | 41.2 | | | | |
| | | | | | | 145 | 35.8 | 1.21 | 42.7 | | | | |
| | | | | | | 144 | 31.4 | 1.14 | 42.8 | | | | |
| | | | | | | 134 | 35.5 | 1.14 | 44.8 | | | | |
| | 14 | | | | | 139 | 38.2 | 1.21 | 40.8 | 150 | 49.8 | 1.43 | 35.6 |
| | | | | | | 162 | 51.5 | 1.53 | 43.4 | 151 | 48.0 | 1.42 | 36.6 |
| | | | | | | 150 | 34.8 | 1.23 | 44.2 | 154 | 41.3 | 1.34 | 44.6* |
| | | | | | | 148 | 35.5 | 1.23 | 52.6 | 150 | 44.2 | 1.36 | 45.8** |
| | 14½ | | | | | | | | | 150 | 44.5 | 1.36 | 40.5** |
| | 14⅔ | | | | | | | | | 154 | 42.7 | 1.36 | 40.0* |
| | 14¾ | | | | | | | | | 157 | 59.0 | 1.59 | 34.1 |
| | 15 | | | | | | | | | 156 | 44.4 | 1.40 | 35.2 |
| | | | | | | | | | | 170 | 56.3 | 1.65 | 37.6 |

*, ** Same subjects respectively.

calculated for surface area (24): Table II, Charts 3, 4) demonstrated a definite increase in the basal metabolic rate immediately before and during pubescence, followed by a corresponding fall after puberty is established.

In a preliminary report Sandiford and Harrington (16) found the decrease in calories per square meter per hour for both boys and girls to be nearly, if not absolutely, a linear function of age. But when first degree

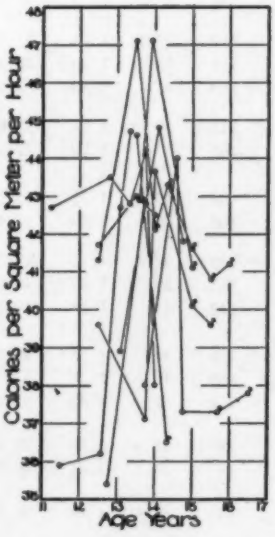


CHART 3

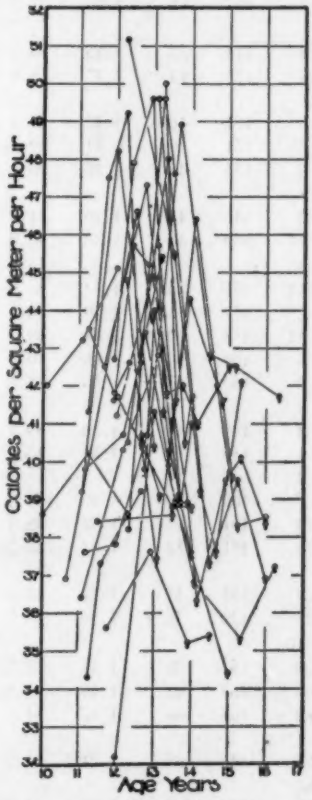


CHART 4

CHART 3. Basal metabolic rate of individual boys (after data of Topper and Mulier). ○ represents boys lacking signs of puberty. ♂ represents boys showing signs of puberty. A prepubertal rise occurs in each subject.

CHART 4. Basal metabolic rate of individual girls (after data of Topper and Mulier). ○ represents pre-pubertal girls. • represents time of first menstruation. ♀ represents post-pubertal girls. The prepubertal trend is upward, the post-pubertal trend downward. The individual peaks precede the first menstruation.

TABLE II

CALORIES PER SQUARE METER OF BODY SURFACE PER HOUR DURING PUBERTY

*(after Topper and Mulier)*

Girls

| Age* | Height | Weight | Area | Rate | Age* | Height | Weight | Area | Rate |
|---|---|---|---|---|---|---|---|---|---|
| yrs:mos. | cm. | kg. | sq. m. | cal. | yrs:mos. | cm. | kg. | sq. m. | cal. |
| 12:0 | 148 | 49.6 | 1.42 | 32.2 | 12:6 | 150 | 49.8 | 1.44 | 43.4 |
| **12:10** | | | | | **13:0** | | | | |
| 13:0 | 154 | 48.9 | 1.45 | 41.3 | 13:7 | 155 | 56 | 1.54 | 38.9 |
| 14:1 | 158 | 56 | 1.56 | 36.8 | 15:0 | 159 | 58.5 | 1.60 | 34.4 |
| 15:4 | 160 | 57 | 1.58 | 35.3 | | | | | |
| 16:3 | 163 | 61 | 1.66 | 37.2 | 13:1 | 161 | 40.9 | 1.38 | 45.7 |
| | | | | | **13:3** | | | | |
| 12:3 | 143 | 33 | 1.16 | 40.5 | 13:7 | 165 | 50.3 | 1.54 | 38.9 |
| 13:4 | 153 | 43 | 1.37 | 48.0 | 14:0 | 167 | 50.5 | 1.56 | 41.0 |
| **13:5** | | | | | | | | | |
| 14:6 | 153 | 46.6 | 1.42 | 37.3 | 12:0 | 157 | 79 | 1.80 | 41.7 |
| 15:3 | 154 | 46 | 1.41 | 39.0 | **13:0** | | | | |
| 16:0 | 155 | 48.7 | 1.46 | 36.9 | 13:6 | 162 | 78 | 1.83 | 38.8 |
| | | | | | 14:0 | 162 | 78 | 1.83 | 38.8 |
| 12:0 | 142 | 41 | 1.26 | 41.2 | | | | | |
| 12:11 | 150 | 46 | 1.38 | 44.9 | 10:8 | 135 | 27 | 1.02 | 36.9 |
| **13:3** | | | | | 12:6 | 147 | 36 | 1.24 | 46.6 |
| 13:11 | 156 | 55 | 1.54 | 38.9 | **12:9** | | | | |
| 14:2 | 157 | 58.6 | 1.59 | 36.3 | 12:11 | 149 | 35.5 | 1.24 | 43.3 |
| 14:11 | 158 | 60.3 | 1.61 | 39.5 | 13:5 | 150 | 40 | 1.30 | 40.3 |
| 15:4 | 160 | 63 | 1.66 | 40.1 | 14:0 | 153 | 41 | 1.33 | 37.6 |
| 16:0 | 160 | 63.4 | 1.66 | 38.4 | | | | | |
| | | | | | 11:9 | 145 | 49 | 1.38 | 35.6 |
| 13:4 | 148 | 41 | 1.30 | 39.9 | 12:11 | 160 | 67 | 1.70 | 37.6 |
| 14:1 | 155 | 44 | 1.40 | 46.4 | **13:1** | | | | |
| **14:2** | | | | | 13:11 | 163 | 65.8 | 1.71 | 35.2 |
| 14:8 | 160 | 50 | 1.50 | 42.0 | 14:6 | 168 | 70 | 1.80 | 35.4 |
| 15:3 | 161 | 51 | 1.52 | 38.3 | | | | | |
| 16:0 | 161 | 52 | 1.53 | 38.5 | 11:3 | 138 | 32.4 | 1.13 | 41.3 |
| | | | | | 11:9 | 142 | 38 | 1.22 | 47.5 |
| 12:9 | 151 | 37 | 1.27 | 39.8 | 12:3 | 145 | 41 | 1.28 | 49.2 |
| 13:8 | 159 | 38.9 | 1.34 | 48.9 | **12:5** | | | | |
| **13:10** | | | | | 12:8 | 150 | 43 | 1.35 | 40.5 |
| 14:6 | 162 | 39.8 | 1.38 | 42.8 | 13:1 | 150 | 46.5 | 1.39 | 37.4 |
| 15:2 | 163 | 42 | 1.41 | 42.5 | 13:6 | 150 | 47 | 1.40 | 41.1 |
| 16:4 | 165 | 46 | 1.47 | 41.7 | | | | | |
| | | | | | 10:0 | 139 | 45.4 | 1.30 | 38.6 |
| 12:3 | 146 | 35 | 1.21 | 51.2 | 11:3 | 142 | 53.2 | 1.41 | 40.2 |
| **13:0** | | | | | **12:1** | | | | |
| 13:6 | 156 | 41 | 1.36 | 45.6 | 12:3 | 152 | 61.6 | 1.58 | 38.6 |
| 14:3 | 162 | 45 | 1.46 | 39.2 | | | | | |
| 14:8 | 168 | 50 | 1.55 | 37.6 | 11:6 | 143 | 57 | 1.47 | 38.4 |
| 15:4 | 163 | 55 | 1.58 | 42.1 | **12:11** | | | | |
| | | | | | 13:6 | 157 | 70.5 | 1.71 | 38.6 |
| 11:8 | 146 | 33.5 | 1.19 | 42.5 | | | | | |
| 12:9 | 155 | 39.5 | 1.33 | 47.3 | 11:3 | 149 | 35 | 1.23 | 43.5 |
| **12:10** | | | | | 11:11 | 154 | 37.7 | 1.29 | 41.8 |
| 13:4 | 158 | 43.5 | 1.41 | 41.8 | 12:4 | 156 | 41 | 1.36 | 42.6 |
| 13:9 | 164 | 45.6 | 1.46 | 42.0 | | | | | |
| 14:2 | 165 | 49 | 1.53 | 41.0 | 11:1 | 139 | 29 | 1.07 | 39.2 |
| 15:0 | 166 | 50.5 | 1.55 | 42.5 | 12:0 | 149 | 34 | 1.21 | 48.2 |
| | | | | | **12:8** | | | | |
| | | | | | 13:0 | 154 | 41 | 1.34 | 40.4 |

TABLE II(*continued*)

| Girls | | | | | Boys | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Age* | Height | Weight | Area | Rate | Age* | Height | Weight | Area | Rate |
| yrs:mos. | cm. | kg. | sq. m. | cal. | yrs:mos. | cm. | kg. | sq. m. | cal. |
| 11:3 | 148 | 48 | 1.40 | 34.3 | 12:6 | 138 | 32 | 1.12 | 41.3 |
| 11:7 | 150 | 49 | 1.42 | 37.3 | 13:6 | 145 | 35.8 | 1.21 | 47.1 |
| 12:8 | 157 | 50 | 1.48 | 39.2 | **14:0** | 146 | 35.2 | 1.21 | 42.1 |
| | | | | | 14:4 | 148 | 38 | 1.27 | 43.3 |
| 11:1 | 145 | 50.5 | 1.40 | 36.4 | 15:0 | 152 | 39.5 | 1.31 | 41.6 |
| 12:2 | 150 | 50 | 1.44 | 40.3 | | | | | |
| 13:1 | 156 | 50 | 1.47 | 42.8 | 13:9 | 165 | 69 | 1.76 | 38.0 |
| | | | | | 14:7 | 168 | 65.2 | 1.75 | 44.0 |
| 12:3 | 156 | 50 | 1.47 | 44.8 | 14:9 | 170 | 63 | 1.74 | 37.3 |
| **12:6** | | | | | **15:8** | 171 | 67.4 | 1.78 | 37.3 |
| 12:8 | 159 | 59 | 1.61 | 40.7 | 16:6 | 174 | 67.5 | 1.81 | 37.8 |
| 13:2 | 160 | 59 | 1.61 | 39.1 | | | | | |
| | | | | | 13:1 | 158 | 41 | 1.37 | 38.9 |
| 12:0 | 140 | 30.4 | 1.10 | 42.7 | 14:1 | 167 | 50 | 1.55 | 44.8 |
| 12:5 | 144 | 36.5 | 1.22 | 47.9 | 14:9 | 170 | 56 | 1.65 | 41.8 |
| 13:0 | 146 | 41 | 1.29 | 49.6 | **15:6** | 171 | 57 | 1.66 | 40.8 |
| **13:1** | | | | | 16:0 | 172 | 55 | 1.65 | 41.2 |
| 13:3 | 148 | 42 | 1.31 | 41.3 | | | | | |
| | | | | | 11:3 | 125 | 29 | 1.00 | 42.7 |
| 11:11 | 136 | 31.5 | 1.10 | 41.6 | 12:10 | 131 | 32 | 1.06 | 43.5 |
| 12:5 | 142 | 33 | 1.16 | 45.7 | 13:4 | 133 | 33 | 1.11 | 42.8 |
| 12:10 | 142 | 35 | 1.18 | 45.2 | 13:9 | 139 | 39 | 1.22 | 44.2 |
| | | | | | 14:0 | 141 | 44 | 1.30 | 38.0 |
| 12:4 | 135 | 32.5 | 1.10 | 38.2 | | | | | |
| 12:8 | 137.5 | 34 | 1.14 | 42.6 | 12:6 | 133 | 34 | 1.11 | 39.6 |
| 13.3 | 140 | 38 | 1.21 | 49.6 | 13:9 | 135 | 36 | 1.16 | 37.1 |
| 13:6 | 142 | 39.5 | 1.25 | 47.6 | 14:0 | 141 | 36.5 | 1.19 | 43.7 |
| | | | | | **15:0** | 149 | 41 | 1.30 | 40.1 |
| 13:0 | 152 | 31 | 1.17 | 49.6 | 15:6 | 151 | 45 | 1.37 | 39.6 |
| **13:1** | | | | | | | | | |
| 13:10 | 155 | 35 | 1.26 | 40.5 | 11:6 | 135 | 50 | 1.33 | 35.9 |
| 14:0 | 155 | 36 | 1.27 | 41.7 | 12:7 | 143 | 49 | 1.38 | 36.2 |
| | | | | | 13:1 | 145 | 50 | 1.40 | 42.7 |
| 10:1 | 130 | 27 | .99 | 42.0 | 13:4 | 147 | 52 | 1.43 | 44.7 |
| 11:1 | 136 | 32 | 1.10 | 43.2 | | | | | |
| 12:0 | 142 | 37 | 1.20 | 45.1 | 12:6 | 119 | 25 | .90 | 41.7 |
| | | | | | 13:6 | 129 | 28 | 1.01 | 43.0 |
| 11:2 | 125 | 31 | 1.03 | 37.6 | 14:0 | 130 | 29 | 1.03 | 42.5 |
| 12:0 | 134 | 32 | 1.09 | 37.8 | | | | | |
| 12:9 | 135 | 36 | 1.16 | 40.7 | 12:9 | 150 | 55 | 1.49 | 35.4 |
| 13:0 | 138 | 40 | 1.23 | 44.0 | 13:8 | 155 | 54 | 1.51 | 42.9 |
| **13:3** | | | | | | | | | |
| 13:8 | 141 | 43 | 1.28 | 39.1 | 13:7 | 132 | 31.5 | 1.07 | 42.9 |
| | | | | | 13:11 | 136 | 31 | 1.09 | 47.1 |
| 13:3 | 150 | 44.6 | 1.36 | 50.0 | **15:0** | 144 | 34 | 1.18 | 41.1 |
| **13:6** | | | | | | | | | |
| 13:7 | 152 | 44 | 1.37 | 41.6 | 13:6 | 148 | 42 | 1.30 | 44.6 |
| 13:11 | 153 | 45.6 | 1.40 | 44.3 | **14:4** | 158 | 49 | 1.48 | 36.5 |
| 14:10 | 157 | 49.5 | 1.47 | 41.6 | | | | | |
| 15:1 | 157 | 49 | 1.47 | 39.6 | | | | | |
| | | | | | | | | | |
| 11:2 | 137 | 30 | 1.08 | 39.8 | | | | | |
| 12:2 | 141 | 32.2 | 1.13 | 40.7 | | | | | |
| **13:2** | 148 | 39.5 | 1.28 | 45.4 | | | | | |

* **Black type figures** signify the onset of menses in the female, and first observation of signs of puberty in the male.

equations are calculated by the method of least squares for the yearly means of the Boothby and Sandiford revision (17) of the Du Bois standards (Table III), the residuals for children show, in contrast to the haphazard scatter of the individual points to either side of the similar fitted straight lines for adults, a distinct tendency toward mounting to positive modes and tailing off negatively (Chart 5). The fitted straight lines for children are transposed with their slopes retained so as to render the re-

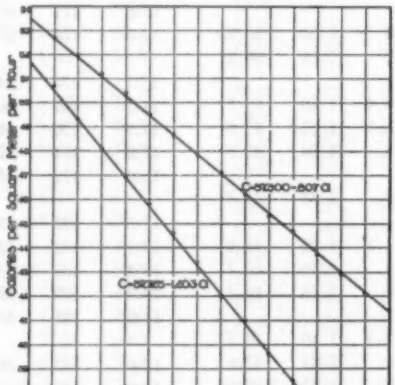


CHART 5. Basal metabolic rate (Boothby and Sandiford). Fitted straight lines. The individual points tend to mount above these lines medially and to fall below them terminally. If the lines were shifted so that all points would fall on or above them, the distances of the individual points above these lines would form the polygons shown in the lower details, to which modal curves are fitted. These curves would then represent cyclic increments. The shifted straight lines would represent the underlying decrease of the basal metabolic rate with age.

siduals positive or zero. Doubly limited, unimodal, skew curves equivalent to the polygons formed by the positive residuals (Chart 5) are calculated by the method of moments (18). The linear equations then represent the decrease of the basal metabolic rate with age, while the binomial equations represent cyclic increments (Table III).

An equation can as readily obscure as typify the actual mode of variation. The basal metabolism is the total exothermal product of the sum of chemical reactions proceeding in the post-absorptive resting organism. The variation of any factor which accelerates the basal metabolism, and

TABLE III
CALORIES PER SQUARE METER OF BODY SURFACE PER HOUR (BOOTHBY AND SANDIFORD)

TABLE III
CALORIES PER SQUARE METER OF BODY SURFACE PER HOUR (BOOTHBY AND SANDIFORD)

| Sex | Discontinuity and range | Equation* | Standard deviation |
|---|---|---|---|
| | yrs. | | cal. |
| Male | (4.5)–20.0 | $C=57.500-0.807a$ | 0.17 |
| | (4.5)–19.0 | $C=57.400-0.807a+0.1986\left(1+\frac{a-9.064}{4.064}\right)^{0.3660}\left(1-\frac{a-9.064}{7.936}\right)^{0.6047}$ | 0.12 |
| | (4.5)–20.6 | $C=58.44^{-0.0170a}$ | 0.24 |
| | (4.5)–20.0 | $C=58.14e^{-0.0171a}+0.4727\left(1+\frac{a-9.405}{3.536}\right)^{0.3722}\left(1-\frac{a-9.405}{8.822}\right)^{0.4897}$ | 0.12 |
| | | $=1+0.009719\left(1+\frac{a-10.28}{4.408}\right)^{0.5425}\left(1-\frac{a-10.28}{7.950}\right)^{0.4272}\Big\}58.14e^{-0.0171a}$ | |
| | 19.0–(79.5) | $C=43.683-0.124a$ | 0.10 |
| | 20.0–(79.5) | $C=44.61e^{-0.0035a}$ | 0.07 |
| Female | (4.5)–16.9 | $C=57.725-1.203a$ | 0.10 |
| | (4.5)–16.0 | $C=57.625-1.203a+0.1603\left(1+\frac{a-8.713}{4.014}\right)^{0.4200}\left(1-\frac{a-8.713}{8.244}\right)^{0.4666}$ | 0.10 |
| | (4.5)–17.2 | $C=59.68e^{-0.0271a}$ | 0.23 |
| | (4.5)–17.0 | $C=59.50e^{-0.0272a}+0.4644\left(1+\frac{a-11.42}{8.390}\right)^{2.778}\left(1-\frac{a-11.42}{4.518}\right)^{1.455}$ | 0.11 |
| | | $=1+0.01036\left(1+\frac{a-11.57}{8.546}\right)^{2.507}\left(1-\frac{a-11.57}{4.362}\right)^{1.432}\Big\}59.50e^{-0.0272a}$ | |
| | 16.0–(79.5) | $C=38.786-0.0824a$ | 0.08 |
| | 17.0–(79.5) | $C=38.98e^{-0.0034a}$ | 0.10 |

* $C$ = calories per square meter per hour, $a$ = age in years, $e$ = natural logarithmic base.

the depletion or withdrawal of which removes this acceleration, must alter the basal metabolic rate in proportion to its present intensity. A fixed decrement per unit time interval cannot therefore be rationally deducted from the basal metabolic rate. The magnitude of the basal metabolic rate is not subject to addition or subtraction. The variation of the basal metabolic rate cannot therefore be represented by linear equations. Irrespective of goodness of fit, linear equations are inappropriate to express the decrease in basal metabolic rate with age.

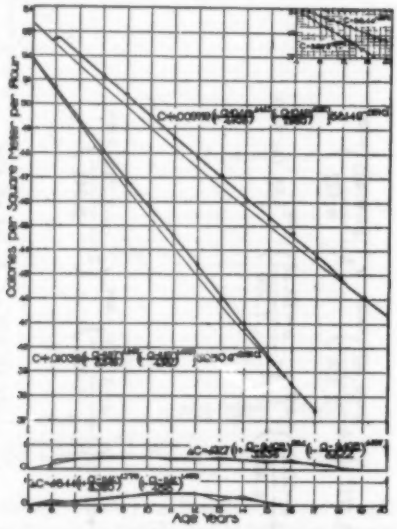


CHART 6. Basal metabolic rate (Boothby and Sandiford). Fitted straight lines on an arithlog grid are shown in the upper right-hand corner detail. The individual points mount above these lines medially and fall below them terminally. The lighter lines on the main chart represent the decrease of the basal metabolic rate with age expressed by exponential equations readjusted so that all points fall on or above them. The modal curves below represent the cyclic increments by which these trends must be increased to meet the actual trends. The heavy lines on the main chart represent the actual trends. In the adjoining equations the cyclic elevations of the basal metabolic rate above the base lines of its decrease are expressed, not as increments, but as accelerations.

The basal metabolic rate per unit surface area may, however, undergo such retardation that the rate of its decrease is constantly proportional to its value. On this basis exponential equations were calculated (19) for the Boothby and Sandiford revision of the Du Bois standards. The residuals for the preadult years themselves vary with age as though a subordinate factor which arose and subsided during this period were neglected (Chart

6). The exponential equations are recalculated to make the residuals positive or zero (Chart 6). Binomial equations for the resulting positive increments are calculated (Table III). The polygons and the fitted curves are plotted (Chart 6). These equations are recalculated to express their ordinates as divided by the ordinates of the exponential equations in order to represent the accelerative factor which elevates the basal metabolic rate above the base line of its decrease (Table III: Chart 6). Graphs of the percentile age distribution of the cyclic accelerative factor in each sex are drawn (Chart 7). These represent the pubertal metabolic acceleration.

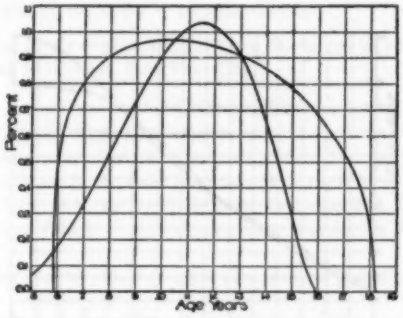


CHART 7. Pubertal metabolic acceleration. The accelerative factor in per cent is plotted against age. The round-peaked graph represents the male, the sharp-peaked the female.

The surface area to which the basal metabolism is referred itself increases at a variable rate. For puberty occurs during a period of growth acceleration. The adolescent growth cycle for weight has a range of from 10 or 11 to 20 years with its mode at 12½ years for females and 15 for males (20, 21). Smoothed curves of growth in surface area computed for children of each sex from the linear formula (22) or modifications of the Lissauer formula (23) based on it are similar to those of growth in weight prior to the pubertal inflection (5). Skew sigmoid curves plotting the values computed from yearly averages by the height-weight formula (24) pass through inflections at 12.3 years for females and 14.5 for males (25). The smoothed curve of all reported actual determinations of surface area plotted against age has its inflection at 14.2 years (26). Surface area during the growing period is related to weight as its two-thirds power times a constant (27). The surface area may then be computed from the weight series (20), and skew sigmoid curves of growth in surface area, and their

derived curves of velocity of growth in surface area, plotted (Chart 8). These latter curves mount gradually to a mode at 12.3 years for females, and, after remaining relatively level prior to 7 years, mount to a mode at 14.4 years for males, and decline toward zero toward 19 and 20 years respectively. The modes of the curves calculated for the pubertal metabolic acceleration occur at 11.6 years for females and 10.3 for males (Table III, Chart 7). These metabolic accelerative modes precede the growth accelerative modes. The modes of the pubertal metabolic acceleration would seem to coincide then rather with sexual maturation than with the modes of the adolescent growth cycle. The metabolic acceleration is

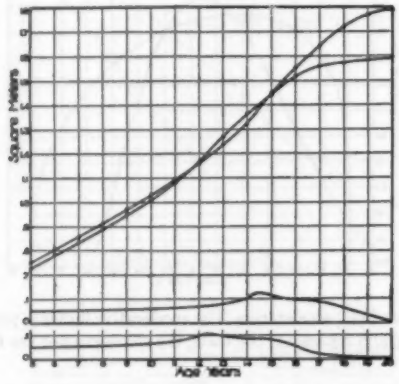


CHART 8. Growth in surface area (calculated from the weight series compiled by Davenport by means of the Boyd and Scammon formula $S = 1.07\ W^{0.575}$ in which $S$ = body surface in square meters, and $W$ = weight in kilos) and rate of growth in surface area. The sigmoid curves represent the total surface area of the body, the modal curves the rate of growth in surface area per year; in each case the upper curve represents the male, the lower the female.

in any case not a result or concomitant of growth. For if a temporary disproportion between the basal metabolism and the surface area arose during the growth cycle, the surface area would preponderate, and the already decreasing basal metabolic rate would be further diminished in relation to the surface area. Growth cannot therefore produce an appearance of a basal metabolic acceleration, but may tend to obscure this acceleration, and lessen or distort the apparent acceleration. The cyclic acceleration in the basal metabolic rate per unit surface area is not dependent on the adolescent growth cycle, but represents a specific acceleration of the basal metabolism associated with puberty.

## Conclusions

1. The basal metabolic rate during later childhood has been shown in numerous confirmatory investigations to give indications of a definite increase, or an irregularity in its decrease, related to puberty.

2. The variation of the basal metabolic rate per unit surface area with age during the pubertal period may therefore be analyzed as the mathematical resultant of a fundamental decrease of the basal metabolic rate with age, and a superimposed cyclic acceleration.

3. This cyclic acceleration of the basal metabolic rate is not a mere concomitant or effect of the adolescent growth cycle, but represents an independent pubertal metabolic acceleration.

Note: Full size blue prints or photostats of these charts can be obtained by writing to Dr. Curtis Bruen, 205 East 78th Street, New York City.

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# EFFECT OF PEARS, PEACHES, APRICOTS, AND DRIED SULFURED APRICOTS ON URINARY ACIDITY

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In a previous paper (5) the effect of fresh Malaga grapes, several kinds of grape juice, grape concentrate, and Thompson seedless and Muscat seeded raisins on the urinary acidity was reported. The effect (6) of figs and smaller amounts of raisins also has been reported. In a subsequent paper (7) the comparative effect of tomato and orange juices has been given. These studies confirmed and considerably extended the earlier information obtained by Blatherwick (2) and Blatherwick and Long (3). In all cases, the above materials exerted a marked basic effect on the urinary acidity. Because of their present general importance in the diet, it also seemed desirable to ascertain the effect of daily ingestion of similar quantities of pears, peaches, apricots, and dried sulfured apricots.

In previous experiments, 1000 cubic centimeter portions of the juices and 1000 gram portions of the fresh fruit, or 300 gram portions of dried fruits were employed. In order that the results might be comparable, equivalent portions were used in the present experiments.

The tests were of twelve days' duration. As in the previous work, each experiment was divided into two consecutive periods. For the first five days each person received only the basal ration. During the next seven days each received, in addition, either 1000 grams of fresh Bartlet pears, or 1260 grams of canned apricots or peaches (including syrup of choice grade), or 300 grams of dried sulfured apricots containing 2140 parts per million of sulfur dioxide (except as stated later). During the last four days the dried apricots were cooked before serving. Three young men subjects were employed in each experiment, except in that with the dried sulfured apricots, where only two participated. All fruit was of choice commercial quality. The basal diet was the same as that described in a previous paper (5).

All analytical determinations were made in duplicate or triplicate, the average of closely agreeing duplicates only being reported. The methods of analysis were those described previously (5). In addition to the urine

analyses, determinations of the ash content, the alkalinity of the soluble ash, the alkalinity of the insoluble ash (1), and the organic acid content (8) were made upon composite samples of the fruits.

## Results and Discussion

As is shown in Tables I to IV, with the basal diet, the pH of the urine generally reached equilibrium at 5.80 to 5.70. However, the equilibrium varied with the individual from pH 5.65 to 6.05. The average range compares well with the corresponding range observed in previous experiments (5, 6, 7). This is interesting, inasmuch as it indicates the extent to which basal reactions may be duplicated with a considerable number of apparently normal human beings. For example, in the various experiments in the general series of studies on urinary acidity, twenty-four different subjects have been used and the range of pH value on the basal diet has been within the above limits.

The effect of the fruits on the pH of the urine varied, ranging from an average change of about 0.7 pH units with pears to a minimum of 1.45 pH units' change with dried sulfured apricots. In all cases there was a marked increase of the pH of the urine following the addition of the fruits to the diet. Considering the pH of the urine on the last days of the experiment and that on the fourth or fifth day, the fresh pears produced an average increase of approximately 0.70 pH unit, the canned peaches approximately 0.85 pH unit, the canned apricots approximately 1.20 pH units, and the dried sulfured apricots an average increase of 1.45 pH units.

The relatively large increase of pH produced by the dried sulfured apricots in comparison with that produced by the unsulfured canned apricots is of interest. Apparently the inorganic sulfur added in the sulfuring process does not greatly reduce the basic effect of the apricot. This was emphasized by the fact that during the sixth, seventh, and eighth days of the diet, the dried apricots were eaten as such and contained about 2140 parts per million of sulfur dioxide, while during the ensuing or last four days of the diet, the dried fruit was cooked at a simmer for thirty minutes so that the final sulfur dioxide content was from 310 to 340 parts per million. On the average, about 85 per cent of the sulfur dioxide was thus removed, yet the additional increase of pH was only 0.35 to 0.45 pH units as compared to the total change of 1.45 pH units.

The average increase of pH of 1.20 units produced by the canned apricots is considerably greater than that of 0.85 pH unit produced by the

peaches; although both the values for the soluble and total alkalinities of the two fruits were quite similar. Likewise the increase of pH produced by the apricots is much greater than the respective ratios of either the soluble alkalinities or the total alkalinities (Table VI). If it is assumed that the

TABLE VI
ASH CONTENT OF FRUIT

| Material | Grams ash per 100 gms. fruit | Alkalinity of soluble ash cc. N/10 acid per 100 gms. | Alkalinity of insoluble ash cc. N/10 acid per 100 gms. | Total alkalinity cc. N/10 acid per 100 gms. |
|---|---|---|---|---|
| Pears | 0.332 | 30.0 | 12.4 | 42.4 |
| Peaches | 0.533 | 48.0 | 11.0 | 59.0 |
| Apricots | 0.466 | 49.5 | 11.5 | 61.0 |
| Dried apricots | 3.293 | 294.0 | 77.0 | 371.0 |

pH is only finally changed by the basic constituents of the ash and that this change is approximately proportional to the alkalinity of the ash, then it appears that the fruits studied in this experiment do not produce a change in pH value strictly proportional to the alkalinity of their ash. However, the previous work (2, 5, 6, 7) has shown that the above assumption appears to generalize the magnitude of the changes of urinary pH produced by the other fruits studied. Further discussion of these relations will be given later. It is worthy of note that alkaline urines were produced by both the canned and dried sulfured apricots.

From the data in Tables I to IV showing the values for total acids and ammonia excreted daily, it is evident that there was a considerable decrease in their concentrations after the fruits were added to the basal diet. For the pears and peaches the concentrations were not finally reduced to such low values as in previous studies; but in both canned and dried sulfured apricots the total acids and ammonia excreted were greatly reduced by the fruit—the urines finally becoming alkaline.

The evaluation of the quantitative relationship between the excretion of acid in excess of fixed bases, as measured by determining the ammonia and titratable acid, and the carbon dioxide binding power of the blood plasma has been developed by Fitz and Van Slyke (4). In the previous study with grapes and grape products, this method of estimating the lowering of the alkaline reserve was employed. It was shown that the basal diet alone lowered the reserve and that the grapes and grape products assisted in securing a high alkaline reserve. Similar results have been secured

with figs, and tomato and orange juices. From a comparison of the data in Tables I to IV with those of the previous studies, it appears that the pears restored the average alkaline reserve to approximately what it had been at the beginning of the basal diet. Apparently the peaches increased the alkaline reserve somewhat above the normal as estimated from the data of the first day of the experiment. However, with both the canned and dried sulfured apricots, the alkaline reserve appeared to be very greatly increased above the normal for the individuals studied, and with approximately the same increase in both instances.

The organic acid contents of the pears, peaches, and the two kinds of apricots were determined by the method of Van Slyke and Palmer (8). The total amounts daily ingested, as cubic centimeters of one tenth normal acid, are given in Table V. The differences of the average daily organic acid titration of the urine with and without the above fruits added to the basal diet are given. From these values the percentage of the organic acids oxidized or otherwise destroyed in the body may be computed.

The average oxidation of the organic acids of the pears was approximately 94.9 per cent; of peaches 94.2 per cent; of canned apricots, 94.9 per cent, and of dried apricots, 94.6 per cent oxidation. For all four fruits the average was approximately 94.7 per cent oxidation. The percentage of organic acid oxidized was similar for all three fruits, although the total amount of organic acids ingested varied greatly according to the fruit used. For example, the organic acid content of the peaches was two and one-third times that of the pears.

For the purposes of comparison it may be recalled that, in the previous studies consisting of sixteen experiments with grapes and grape products, an average of 94.4 per cent of the organic acids expressed as tartaric acid was oxidized or destroyed. Averages of 97.5 per cent of the organic acids of the figs, 90.7 per cent of the organic acids of tomato juice, and 93.8 per cent of the organic acids of orange juice were oxidized. Apparently the oxidation or destruction of the organic acids by the body proceeded to approximately the same extent for the fruit materials investigated. This is particularly true of grapes and grape products, pears, peaches, canned apricots, and dried sulfured apricots.

## Summary

Experiments with men subjects on a basal diet and on the same basal supplemented by pears, peaches, apricots, and dried sulfured apricots are reported. The following results were observed when the fruits were added to the basal ration.

1. An average increase of the urinary reaction of approximately 0.70 pH unit was produced by 1000 grams fresh Bartlet pears; an average of 0.85 pH unit by 1260 grams of canned peaches and by an equal quantity of canned apricots, an average increase of 1.20 pH units. An average increase of 1.45 pH units was produced by 300 grams of dried sulfured apricots. Both kinds of apricots produced alkaline urines.

2. Corresponding decreases in both the ammonia excreted and in the total acidity were noted. The average changes produced by both kinds of apricots were considerably larger than those resulting from either the peaches or pears.

3. There was an increase of the alkaline reserve, estimated according to the method of Fitz and Van Slyke, equal to or above the normal for each subject. This increase was very marked for both kinds of apricots.

4. There appeared to be a correlation between the alkalinity of the ash and the reaction of the urine in the case of pears and peaches. A more basic reaction was associated with a higher ratio of soluble alkalinity to insoluble alkalinity of the ash. Compared with the peaches, the apricots appeared to produce an even more basic reaction with approximately the same ratio of soluble alkalinity to insoluble alkalinity.

5. A slight increase occurred in the organic acids excreted when pears, peaches, or the above two kinds of apricots were added to the basal ration.

6. The average percentage oxidations of the organic acids of the pears, peaches, and the two kinds of apricots were similar, averaging 94.7 per cent.

7. Apparently the added inorganic sulfur in the apricots does not markedly reduce the basic effect of the apricot on urinary acidity.

The writer wishes to express his appreciation of the interest and advice of Dr. W. V. Cruess, upon whose suggestion this study was initiated. The full co-operation of the men taking a part in the diets is gratefully acknowledged.

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TABLE I

EFFECT OF FRESH PEARS ON COMPOSITION OF URINE

| Days* | Subject S | | | | | Subject D | | | | | Subject M | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Volume | pH | 0.1-N organic acids | 0.1-N titratable acidity | Ammonia N | Volume | pH | 0.1-N organic acids | 0.1-N titratable acidity | Ammonia N | Volume | pH | 0.1-N organic acids | 0.1-N titratable acidity | Ammonia N |
| | cc. | | cc. | cc. | mg. | cc. | | cc. | cc. | mg. | cc. | | cc. | cc. | mg. |
| 1 | 870 | 6.35 | 540 | 85 | 85 | 1270 | 6.10 | 555 | 175 | 85 | 880 | 6.35 | 538 | 134 | 86 |
| 2 | 1100 | 6.15 | 537 | 178 | 96 | 1150 | 6.25 | 518 | 221 | 121 | 1010 | 6.20 | 623 | 164 | 96 |
| 3 | 1280 | 6.00 | 567 | 177 | 116 | 1460 | 6.20 | 584 | 219 | 123 | 800 | 5.80 | 605 | 148 | 112 |
| 4 | 1050 | 5.85 | 502 | 162 | 153 | 1210 | 5.90 | 500 | 214 | 167 | 790 | 5.75 | 560 | 160 | 162 |
| 5 | 1050 | 5.90 | 526 | 145 | 137 | 1070 | 5.85 | 530 | 211 | 170 | 780 | 5.80 | 527 | 146 | 146 |
| 6 | 1330 | 6.20 | 553 | 194 | 149 | 1140 | 6.05 | 551 | 176 | 136 | 780 | 6.00 | 527 | 156 | 147 |
| 7 | 1460 | 6.45 | 584 | 169 | 143 | 1140 | 6.15 | 596 | 204 | 122 | 760 | 6.00 | 601 | 175 | 125 |
| 8 | 1040 | 6.55 | 610 | 164 | 95 | 1040 | 6.20 | 611 | 200 | 138 | 930 | 6.20 | 680 | 188 | 126 |
| 9 | 1120 | 6.75 | 675 | 95 | 94 | 1020 | 6.30 | 651 | 168 | 127 | 840 | 6.30 | 620 | 179 | 103 |
| 10 | 1580 | 6.75 | 616 | 104 | 108 | 1080 | 6.25 | 588 | 160 | 106 | 900 | 6.35 | 594 | 150 | 102 |
| 11 | 1860 | 6.75 | 676 | 86 | 117 | 1170 | 6.35 | 588 | 158 | 85 | 1050 | 6.40 | 660 | 133 | 92 |
| 12 | 1500 | 6.75 | 606 | 72 | 90 | 1070 | 6.40 | 525 | 129 | 71 | 980 | 6.40 | 599 | 106 | 84 |

* Days, 1–5 inclusive, basal diet. Days, 6–12 inclusive, basal + 1000 grams fresh pear.

TABLE II

EFFECT OF CANNED PEACHES ON COMPOSITION OF URINE

| Days* | Volume | pH | 0.1-N organic acid | 0.1-N titratable acidity | Ammonia N | Volume | pH | 0.1-N organic acid | 0.1-N titratable acidity | Ammonia N | Volume | pH | 0.1-N organic acid | 0.1-N titratable acidity | Ammonia N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Subject A | | | | | Subject B | | | | | Subject D | | | | |
| | cc. | | cc. | cc. | mg. | cc. | | cc. | cc. | mg. | cc. | | cc. | cc. | mg. |
| 1 | 880 | 6.45 | 740 | 123 | 173 | 850 | 6.00 | 530 | 192 | 119 | 750 | 6.15 | 378 | 106 | 126 |
| 2 | 1100 | 6.25 | 725 | 105 | 185 | 1210 | 6.00 | 620 | 220 | 203 | 880 | 6.00 | 503 | 148 | 148 |
| 3 | 860 | 5.70 | 675 | 172 | 187 | 860 | 5.65 | 590 | 238 | 272 | 840 | 5.90 | 492 | 215 | 188 |
| 4 | 1120 | 5.70 | 520 | 197 | 219 | 750 | 5.70 | 565 | 246 | 252 | 900 | 5.70 | 486 | 202 | 176 |
| 5 | 1220 | 5.65 | 573 | 181 | 206 | 1240 | 5.70 | 610 | 229 | 261 | 870 | 5.65 | 467 | 195 | 171 |
| 6 | 1080 | 5.75 | 674 | 222 | 181 | 1000 | 5.95 | 672 | 162 | 196 | 660 | 5.75 | 512 | 172 | 166 |
| 7 | 2000 | 6.30 | 650 | 132 | 168 | 1730 | 6.10 | 660 | 125 | 145 | 910 | 6.10 | 523 | 130 | 153 |
| 8 | 1670 | 6.35 | 855 | 134 | 164 | 1580 | 6.10 | 770 | 158 | 177 | 1090 | 6.10 | 654 | 153 | 152 |
| 9 | 1320 | 6.40 | 713 | 132 | 148 | 1270 | 6.10 | 684 | 153 | 178 | 960 | 6.20 | 590 | 128 | 135 |
| 10 | 2120 | 6.40 | 800 | 67 | 89 | 1750 | 6.25 | 744 | 84 | 121 | 910 | 6.25 | 742 | 109 | 115 |
| 11 | 1700 | 6.45 | 857 | 75 | 95 | 1670 | 6.35 | 808 | 94 | 140 | 910 | 6.40 | 810 | 109 | 102 |
| 12 | 1840 | 6.50 | 885 | 63 | 77 | 1620 | 6.45 | 837 | 78 | 91 | 930 | 6.60 | 876 | 82 | 91 |

* Days, 1–5, inclusive, basal diet. Days, 6–12, inclusive, basal+1260 grams canned peaches and juice.

TABLE III

EFFECT OF CANNED APRICOTS ON COMPOSITION OF URINE

| Days* | Volume | pH | 0.1-N organic acids | 0.1-N titratable acidity | Ammonia N | Volume | pH | 0.1-N organic acids | 0.1-N titratable acidity | Ammonia N | Volume | pH | 0.1-N organic acids | 0.1-N titratable acidity | Ammonia N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Subject C | | | | | Subject O | | | | | Subject S | | | | |
| | cc. | | cc. | cc. | mg. | cc. | | cc. | cc. | mg. | cc. | | cc. | cc. | mg. |
| 1 | 980 | 6.20 | 395 | 62 | 127 | 860 | 6.10 | 495 | 112 | 193 | 1350 | 6.35 | 567 | 43 | 151 |
| 2 | 1200 | 6.20 | 625 | 60 | 135 | 760 | 6.15 | 434 | 94 | 171 | 960 | 6.25 | 618 | 99 | 188 |
| 3 | 810 | 5.90 | 541 | 195 | 258 | 810 | 6.05 | 590 | 146 | 182 | 1070 | 6.00 | 643 | 244 | 240 |
| 4 | 1330 | 5.90 | 468 | 187 | 262 | 1030 | 6.00 | 530 | 165 | 202 | 890 | 6.00 | 419 | 244 | 206 |
| 5 | 1040 | 5.90 | 432 | 203 | 263 | 690 | 6.00 | 527 | 159 | 193 | 880 | 6.05 | 450 | 257 | 222 |
| 6 | 980 | 6.30 | 451 | 110 | 247 | 1280 | 6.10 | 548 | 159 | 179 | 850 | 6.15 | 530 | 85 | 185 |
| 7 | 490 | 6.65 | 482 | 63 | 144 | 1110 | 6.65 | 591 | 142 | 170 | 1220 | 6.85 | 595 | 93 | 169 |
| 8 | 1790 | 6.80 | 516 | 72b | 75 | 1650 | 6.75 | 731 | 40b | 116 | 1680 | 6.80 | 692 | 27b | 94 |
| 9 | 1820 | 7.10 | 828 | 77b | 76 | 1550 | 6.85 | 670 | 81b | 108 | 1370 | 7.10 | 695 | 66b | 96 |
| 10 | 1550 | 7.10 | 861 | 81b | 65 | 1700 | 6.80 | 758 | 75b | 95 | 770 | 7.10 | 812 | 75b | 86 |
| 11 | 1530 | 7.15 | 842 | 86b | 64 | 1210 | 7.10 | 842 | 76b | 85 | 1250 | 7.10 | 828 | 83b | 70 |
| 12 | 1580 | 7.20 | 893 | 77b | 66 | 1370 | 7.15 | 814 | 82b | 77 | 1390 | 7.15 | 857 | 86b | 68 |

* Days, 1–5 inclusive, basal diet. Days, 6–12 inclusive, basal+1260 grams canned apricots and juice. b=denotes basic urines.

TABLE IV

EFFECT OF DRIED SULFURED APRICOTS ON COMPOSITION OF URINE

| Days* | Volume | pH | 0.1-N organic acids | 0.1-N titratable acidity | Ammonia N | Volume | pH | 0.1-N organic acids | 0.1-N titratable acidity | Ammonia N |
|---|---|---|---|---|---|---|---|---|---|---|
| | Subject H | | | | | Subject T | | | | |
| | cc. | | cc. | cc. | mg. | cc. | | cc. | cc. | mg. |
| 1 | 1080 | 6.00 | 462 | 151 | 121 | 660 | 6.40 | 465 | 122 | 129 |
| 2 | 850 | 5.95 | 588 | 172 | 167 | 960 | 5.95 | 610 | 173 | 161 |
| 3 | 870 | 6.00 | 535 | 174 | 195 | 990 | 6.05 | 594 | 171 | 193 |
| 4 | 890 | 5.90 | 431 | 199 | 199 | 1040 | 5.70 | 495 | 239 | 204 |
| 5 | 1460 | 5.70 | 494 | 187 | 204 | 850 | 5.65 | 492 | 232 | 214 |
| 6 | 730 | 5.75 | 461 | 170 | 204 | 720 | 5.65 | 504 | 166 | 202 |
| 7 | 1080 | 6.60 | 707 | 58 | 91 | 600 | 6.45 | 508 | 77 | 109 |
| 8 | 1180 | 6.70 | 854 | 28b | 83 | 970 | 6.75 | 748 | 36b | 81 |
| 9 | 1340 | 6.75 | 828 | 32b | 75 | 1080 | 6.75 | 758 | 43b | 91 |
| 10 | 900 | 6.85 | 760 | 65b | 63 | 920 | 6.80 | 762 | 55b | 77 |
| 11 | 820 | 6.85 | 780 | 66b | 69 | 640 | 6.85 | 723 | 42b | 72 |
| 12 | 1020 | 7.15 | 823 | 69b | 57 | 880 | 7.10 | 811 | 58b | 62 |

* Days, 1–5 inclusive, basal diet. Days, 6–12 inclusive, basal+300 grams dried sulfured apricots. b denotes basic urines.

TABLE V

ORGANIC ACIDS INGESTED AND OXIDIZED

(As cc. N/10 HCl)

| | Pears | | | Peaches | | | Apricots | | | Dried Apricots | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | S | D | M | A | B | D | C | O | S | H | T |
| Average daily organic acid titration for basal diet period | 534 | 537 | 571 | 647 | 583 | 465 | 492 | 515 | 539 | 502 | 531 |
| Average daily organic acid titration for basal diet plus fruit | 617 | 587 | 611 | 776 | 739 | 672 | 696 | 708 | 715 | 745 | 688 |
| Difference | 83 | 50 | 40 | 129 | 156 | 207 | 204 | 193 | 176 | 243 | 157 |
| Organic acids ingested daily from fruit | 1140 | 1140 | 1140 | 2840 | 2840 | 2840 | 3780 | 3780 | 3780 | 3690 | 3690 |
| Per cent oxidation of organic acids | 92.8 | 95.6 | 96.5 | 95.5 | 94.5 | 92.7 | 94.6 | 94.9 | 95.3 | 93.4 | 95.7 |

# THE IODINE CONTENT OF HENS' EGGS AS AFFECTED BY THE RATION*

*By*

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THE increasing use of eggs in the human dietary necessitates a more complete knowledge of the variation in the amounts of the different essential components of the egg. Both the poultryman and the consumer are interested in eggs possessing a maximum nutritive value. The poultryman is also devoting his efforts to the production of eggs which contain the maximum amounts of the essential elements and compounds required for the development of strong, sturdy chicks.

A great deal of work has been done on the variations in the amounts of the organic entities of eggs (1). As a result of these investigations it has been definitely established that the vitamin A and vitamin D content of eggs can be greatly influenced by the ration of the bird. Work on the variation in the amounts of the minor inorganic elements in the egg has been given but little consideration. Elvehjem, Kemmerer, Hart, and Halpin (2) reported that neither the iron nor the copper content of eggs can be increased by feeding additional iron or copper. Unpublished work at this Station confirms these results for iron.

The iodine content of eggs, like that of other natural food products, appears to be variable. Forbes and co-workers (3) found traces of iodine in eggs. Hercus and Roberts (4) were able to increase the iodine content of eggs by iodine feeding. Simpson and Strand (5) increased the iodine value of eggs from 0.018 mg. to 0.754 mg. per 100 gm. of shell-free egg, by progressively increasing the dose of potassium iodide to 8 mg. daily over a period of four months. Within two weeks after the iodine feeding was discontinued, the iodine content of the egg dropped to 0.08 mg. per 100 gram of shell-free egg.

The common use of eggs in the human dietary, especially for infants and invalids, and the rather extensive use of iodine in the treatment of endemic goiter, make it desirable that we have further information on the

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extent to which the iodine content of eggs is affected by the iodine intake of the bird.

## Experimental

The initial experiment was in the nature of a preliminary trial to determine the comparative effects of different sources of iodine. Dried kelp, generally recognized as a potent source of iodine, fish meal, and potassium iodide were used. Chemical analysis of the kelp and fish meal used in this experiment showed that they contained 0.135 and 0.00124 per cent iodine, respectively.

Forty White Leghorn hens were divided into four lots of ten birds each and confined indoors. All lots received a basal ration of ground yellow corn 50, ground wheat 15, wheat bran 10, meat scraps 7.5, dried skim milk 5, alfalfa-leaf meal 5, bone meal 2, fine oyster shells 4, cod-liver oil 1, and salt 0.5. The birds were trapnested and eggs from individual hens were analyzed for their iodine content. After sufficient iodine determinations had been made, while the birds were on the basal ration, the rations were modified as follows:

Lot 1. Continued on basal ration.
Lot 2. Basal ration plus potassium iodide.
Lot 3. Basal ration plus 2 per cent kelp.
Lot 4. Basal ration in which meat scraps were replaced by an equivalent amount of Menhaden fish meal protein.

The quantity of potassium iodide added in Lot 2 was such as to make the iodine content of the ration comparable with that fed Lot 3 (kelp). All rations were fed *ad libitum*—no attempt was made to keep feed consumption records. The eggs from individual hens in the different lots were analyzed for iodine by a modification of the Kendall (6) method. While the regular method gave good results in the analysis of feed samples, it was found necessary to introduce certain minor modifications, in order to obtain satisfactory results in the analysis of eggs. An approximate 30 gram sample of egg was used. The method was checked frequently by the recovery of known amounts of iodine in the form of potassium iodide added to egg samples. Of 57 such determinations an average of 96.2 per cent recovery of iodine was obtained.

The results summarized in Table I show that the iodine content of eggs can be increased by increasing the iodine intake of the bird. The increase in iodine value was more or less proportional to the amount of iodine in the ration.

The results suggested that a more comprehensive study of the problem was desirable. The iodine-bearing substances had previously been mixed with the basal ration and fed *ad libitum*, with no check or information on the actual amount of iodine consumed per bird. Accordingly, in the next experiment, the birds were individually fed known amounts of iodine daily.

TABLE I

IODINE CONTENT OF EGGS

(Results expressed in parts per million of fresh, shell-free egg)

| Average of all eggs before feeding iodine | Lot 1 Basal ration | Lot 2 Basal ration plus KI | Lot 3 Basal ration plus kelp | Lot 4 Basal ration with fish meal |
|---|---|---|---|---|
| 0.56 | 0.57 | 8.25 | 7.81 | 0.77 |

Eighteen White Leghorn pullets were confined in individual cages, provided with wire floors, and fed the same basal ration as in the first trial. Individual egg records were kept and all eggs properly marked for identification. Eggs were saved for analysis after the birds had been on the experimental ration for seven days. After sufficient eggs had been saved for analytical purposes, the birds were divided into 3 lots of six birds each. The same basal ration was fed to all lots. In addition Lot 1 received dried kelp; Lot 2 iodized linseed meal[1] and Lot 3 potassium iodide evaporated on dextrin. These products were fed in gelatin capsules in such amounts that each bird received 2 mg. of iodine daily.

After the birds had been on these rations for four weeks the kelp feeding in Lot 1 was discontinued, and the amount of iodine in the form of iodized linseed meal and potassium iodide was increased to 5 mg. daily, in the case of Lots 2 and 3.

Iodine determinations were made on composite homogeneous egg samples from individual birds covering a period of one week. All determinations were made in duplicate or triplicate. The method was checked each day by recovery of known amounts of iodine added to samples of egg. Analyses were made on all eggs produced during the week prior to supplemental iodine feeding, and on those laid during the second and fourth weeks of periods 2 and 3, when the birds received 2 and 5 mg. of iodine daily, respectively. The results are recorded in Table II.

[1] We are indebted to Dr. G. M. Karns, of the Mellon Institute of Industrial Research, Pittsburgh, Pa., for the iodized linseed meal.

TABLE II

THE EFFECT OF IODINE INTAKE ON THE IODINE CONTENT OF EGGS

(Results expressed in parts per million of shell-free fresh egg)

| Lot No. | Hen No. | Weekly periods | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | | Basal ration | | 2 mg. iodine fed daily Kelp | | | | No supplementary iodine feeding (Basal ration only) | | | |
| I | 1 | | ♯ | | 6.42 | | 7.80 | | 0.66 | | 0.25 |
| | 3 | | ♯ | | 7.20 | | 7.20 | | 0.69 | | 0.25 |
| | 4 | | ♯ | | 5.14 | | 10.42 | | 0.30 | | * |
| | 6 | | ♯ | | * | | 4.94 | | 0.78 | | * |
| | 7 | | ♯ | | 4.68 | | * | | 0.36 | | 0.25 |
| | 16 | | * | | * | | * | | * | | * |
| | Av. | | | | 5.86 | | 7.59 | | 0.55 | | 0.25 |
| | | Basal ration | | 2 mg. iodine fed daily Iodized linseed meal | | | | 5 mg. iodine fed daily Iodized linseed meal | | | |
| II | 8 | | ♯ | | 6.34 | | 4.22 | | * | | * |
| | 9 | | ♯ | | 8.31 | | 8.01 | | 17.17 | | 14.74 |
| | 11 | | ♯ | | 6.74 | | 11.40 | | 16.57 | | 19.97 |
| | 12 | | ♯ | | 3.37 | | 6.00 | | 11.22 | | 11.11 |
| | 13 | | ♯ | | 5.02 | | 7.88 | | 9.74 | | 10.60 |
| | 15 | | ♯ | | 5.54 | | 5.85 | | 13.05 | | 15.17 |
| | Av. | | | | 5.89 | | 7.37 | | 13.55 | | 14.31 |
| | | Basal ration | | 2 mg. iodine fed daily potassium iodide | | | | 5 mg. iodine fed daily potassium iodide | | | |
| III | 5 | | ♯ | | 5.62 | | 7.62 | | 12.74 | | 14.85 |
| | 10 | | ♯ | | 4.80 | | 6.60 | | 11.88 | | 12.51 |
| | 14 | | * | | * | | * | | * | | * |
| | 17 | | ♯ | | 7.42 | | 7.54 | | 13.37 | | 23.02 |
| | 18 | | ♯ | | 6.80 | | 9.34 | | 14.80 | | 20.77 |
| | 20 | | ♯ | | 6.88 | | 12.94 | | 15.14 | | 13.22 |
| | Av. | | | | 6.30 | | 8.80 | | 13.58 | | 16.87 |

♯ Approximately 0.1 part per million.

* No eggs laid during week.

The data show conclusively that the iodine content of the egg is dependent upon and directly related to the iodine intake of the bird. These findings are in agreement with those of Hercus and Roberts (4) and Simpson and Strand (5). The per cent of iodine in the egg rises rapidly after iodine feeding, but apparently does not reach its maximum value until sometime after 2 weeks of supplemental feeding. The decline in iodine content is equally rapid after cessation of its feeding, but, like the increase, it required more than 2 weeks to reach again a minimum value.

The percentage of iodine in the egg was independent of the source of iodine when equivalent amounts of this element were fed. Apparently iodine in organic combination, as in kelp, is utilized no more efficiently than in the inorganic state. Eggs produced on the unsupplemented basal ration contained approximately 0.1 part of iodine per million. This amount, in all probability, was chiefly derived from the cod-liver oil in the ration. The feeding of 2 mg. of iodine daily, either as kelp, iodized linseed meal, or potassium iodide, increased the iodine content of the egg approximately 75 times. When the level of iodized linseed meal and potassium iodide was increased to 5 mg. of iodine daily, the iodine percentage of the egg was increased about 150 times.

No attempt was made to study the effects of an increased iodine content of the egg on hatchability, or the livability of chicks hatched from such eggs.

Whether eggs of a known iodine content have a particular place in human nutrition probably depends upon whether the iodine requirements of the people of a given locality are provided from other sources. It is common knowledge that the natural foods and drinking water of certain sections are deficient in iodine, and that some form of medication must be resorted to in order to prevent and control endemic goitre. How this can be best accomplished remains an open question. It would seem that medication through the use of natural foods, whose iodine content has been raised by administering the element to the animal, bird, or plant producing them, as suggested by Weston (7), Maurer (8), and Zickgraf (9) would in general be less hazardous and more desirable than by other means.

## Summary

1. The iodine content of hens' eggs varies directly with the amount of this element in the ration of the bird.

2. The feeding of 2 and 5 mg. of iodine daily per bird, in the form of

dried kelp, iodized linseed meal, or potassium iodide, increased the iodine content of the eggs approximately 75 and 150 times, respectively.

3. The percentage of iodine in eggs immediately decreases upon the discontinuance of iodine feeding.

4. The amount of iodine in eggs is independent of the form in which it is fed to the birds.

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